Introduction

In living organisms, there are a group on compounds, carbohydrates, which play a vital role in the metabolism and functioning of that organism. The main use for carbohydrates is that they provide sources of energy to that organism. These carbohydrates are stored in organisms as polysaccharides, which is the polymerization of many sugars, like glycogen and starch.

There are two main structures of carbohydrates of either heteropolymers or homopolymers. Homopolymers with unusual linkages, [β(1→4) or α(1→6)], examples of this are cellulose and dextran respectively. Heteropolymers consists of sugars, amino sugars, uronic acids etc. Examples of heteropolymerized carbohydrates are hyaluronic acid and chondroitin.

In this investigation, carbohydrates will be worked with and separated based on their molecular size. This first thing that will be done is a dialysis with a starch-glucose solution. This separation occurs because the glucose can pass through the semi-permeable membrane while the starch has a tougher time doing so. The second method of separation that will be preformed is gel filtration chromatography. This separation uses hydrated beads with a highly controlled porosity. The solution is placed in the column with an aqueous buffer. The molecules are then separated biggest to smallest. After this separation is done the solution will be separated into different fractions.

Once the separations have been completed, a glucose test was performed on specific fractions of the solution. Glucose Oxidase-peroxidase will be used for this reaction. This first test will test for the presence of glucose in specific fractions, where the glucose will be proposed to be. Similarly, and iodine test will be done to test for the starch in the fractions that are expected to have starch.

Experimental Procedure

Refer to pages 11-14 in 'BCH 2333: Introduction to Biochemistry Laboratory Component' manual.

Observations

Important Steps	Observations
Experiment #1: Dialysis	• When the dialysis tube filled with 10 mL of starch glucose solution was placed into the beaker filled with 200 mL of water, diffusion was occurring.
Experiment #2: Gel Filtration Chromatography	• After 55 minutes had elapsed, the dialysis bad was taken out of the beaker, both solutions were still colorless • Once the solution was loaded, 30 fractions of 90 drops were collected.
Experiment #3: Glucose Oxidase	• Various tests were performed on the assayed solution, glucose oxidase-peroxidase and different sugar solutions including the internal and external contests from the dialysis experiment • When the 2N H_2SO_4 was added to each test tube, some of the solutions changed color while others seemed to stay colorless.

Experiment #4: Iodine Reaction	• Various tests were performed on the assayed solution, iodine and different sugar solutions including the internal and external contests from the dialysis experiment • When the iodine was added to the fractions, certain test tubes change color while others stayed the same.

Results and Discussion – Properties

1.

Table #1: Carbohydrates
Summarization of the Iodine and Glucose Oxidase Tests

Reagent	Glucose Oxidase	Iodine
Glucose	Positive	Negative
Starch	Negative	Positive
Sucrose	Negative	Negative
Fructose	Negative	Negative
Dialysis: Internal Solution	Positive	Positive
Dialysis: External Solution	Positive	Negative

Iodine and Glucose Oxidase tests were performed according to the experimental procedure, in the laboratory manual.

Form the table above, it can be seen that glucose will test positive with the glucose oxidase test while test negative for the iodine test. This is true because glucose has a free aldose that can be oxidizes. As for the iodine test, glucose is a composed of a single sugar therefore the iodine molecules cannot bind to the sugar.

Starch is a composed of many sugar monomers. For this reason, starch tests negative for the glucose oxidase test. There is no free aldose or ketose group that can be reduced. In terms on the iodine test, a starch solution would test positive because there is areas in which the iodine molecules can bind to the sugar.

Sucrose tests would test negative in the glucose oxidase test because of the fact that there is no free aldose or ketose that can be reduced. On the other hand sucrose would test negative for the iodine test because even though it is a disaccharide, the sucrose molecule is not that big. This means that the iodine molecules would still have a hard time binding to the sucrose.

If fructose was to be tested with glucose oxidase or iodine it would text negative for both of these tests. The reason why fructose would test negative for both of these tests is becuase fructose is composed of just a single sugar molecule, a monosaccharide. This would mean that the iodine molecules would not be able to bind to the fructose. As mentioned, fructose would also fail the glucose oxidase test because the glucose oxidase enzyme is glucose specific and will only give a positive test if there is glucose present in the solution that is being tested.

The internal contents tested positive for both the starch and the glucose. The internal contents of this tube tested positive for both starch and glucose because the dialysis tube is a semi-permeable membrane. This means that smaller molecules can go through the membrane while larger molecules cannot. This means that when the dialysis tube was taken out of the water there was still some glucose left in the tube, while almost all of the starch was still in the tube. For this reason that the glucose could go freely in and out of the dialysis tube, the external contents tested positive for glucose while texted negative for the starch.

Dialysis

2.

Table #2: Dialysis Results

Summarization of Dialysis Results from Experiment #1

t (min)	Glucose				Starch			
	A_{420} Int soln.	c_1 (g/L)	A_{420} Ext soln.	c_2 (g/L)	A_{600} Int soln.	c_1 (g/L)	A_{600} Ext soln.	c_2 (g/L)
0	----	50	----	0	----	1	----	0
55	0.41	11.23	0.31	16.12	0.79	0.967	0.035	0.188

The above data shown was taken from the results of experiment #1, while the concentrations were calculated from the formula, $A_1c_1 = A_2c_2$ where A is absorbance and c is concentration.

3. In our investigation, the volume of the dialysis bag did slightly get altered; the initial volume was 10.0 mL while the final volume was 10.3 mL. In theory, the volume of the dialysis bag should not have been changed because the net change in molecules that are exchanged during the diffusion process is little to none. The reason why there is a small fluctuation between the initial and final volume is due to experimental errors with the transferring the contents of the dialysis tube from the beaker to the graduated cylinder. The main error here is the liquid that was dripping from the tube and hands transferring the tube, some may have gotten into the graduated cylinder.

4. $a = \frac{V_1}{V_2} \leftrightarrow \frac{10\ mL}{200\ mL} = 0.05$

$$c_{eq} = \frac{c_o a}{(1 + a)}$$

$$c_{eq} = \frac{\left(50\frac{g}{L}\right)(0.05)}{(1 + 0.05)}$$

$$c_{eq} = 2.38\ g/L$$

5.

First we have to find β, using equation 2:

$$\begin{aligned}
\left(c_1 - c_{eq}\right)(1 + \alpha) &= c_o e^{-\beta t} \\
(11.23 - 2.38)(1 + 0.05) &= 50e^{-\beta(55)} \\
9.2925 &= 50e^{-\beta(55)} \\
0.18585 &= e^{-\beta(55)} \\
\ln(0.18585) &= -\beta(55) \\
\beta &= 0.0305
\end{aligned}$$

Where:

- c_1 = 11.23 g/L
- c_{eq} = 2.38 g/L
- α = 0.05
- c_o = 50 g/L
- t = 55 mins
- β = ?

Second we calculate for D, using equation 4:

$$\beta = \frac{2(1+\alpha)D}{r^2}$$
$$0.0305 = \frac{2(1+0.05)D}{0.8^2}$$

$$D = \frac{(0.0305)(0.8^2)}{2(1+0.05)}$$
$$D = 0.0092$$

Where:

- β = 0.0305 min^{-1}
- α = 0.05
- r = 0.8 cm
- D = ?

6.

$$\left(c_1 - c_{eq}\right)(1+\alpha) = c_o e^{-(0.0305)t}$$
$$(12.5 - 2.38)(1+0.05) = 50e^{-(0.0305)t}$$
$$(12.5 - 2.38)(1+0.05) = 50e^{-(0.0305)t}$$
$$10.626 = 50e^{-(0.0305)t}$$
$$0.2125 = e^{-(0.0305)t}$$
$$\frac{\ln(0.2125)}{-0.0305} = t$$
$$t = 50.77\ mins$$

Where:

- c_1 = 12.5 g/L
- c_{eq} = 2.38 g/L
- α = 0.05
- c_o = 50 g/L
- t = ?
- β = 0.0305 min^{-1}

Gel Filtration

7.

Table #3: Column Elution Results

Summarization of Absorbance's and Concentrations of Experiments 3 and 4

Fraction		**Glucose**		**Starch**	
Test tube #	**Elution V (mL)**	**A_{420}**	**C (g/L)**	**A_{600}**	**C (g/L)**
7	9.8	----		0.024	0.0046
8	11.2			0.027	0.0052
9	12.6			0.019	0.0038
10	14.0			0.177	0.0342
11	15.4			0.784	0.1500
12	16.8			0.220	0.0425
13	18.2			0.042	0.0080
14	19.6			0.036	0.0070
15	21.0	0.015	0.411	----	
16	22.4	0.036	0.986		
17	23.8	0.029	0.795		
18	25.2	0.032	0.877		
19	26.6	0.054	1.479		
20	28.0	0.079	2.164		
21	29.4	0.048	1.320		
22	30.8	0.028	0.767		
23	32.2	0.025	0.685		
24	33.6	0.028	0.767		

25	35.0	0.036	0.986	

The above data shown summarizes the results obtained from experiments 3 and 4. The concentrations were calculated by the formula; $A = \varepsilon c l$

Sample Calculation:

$$A = \varepsilon c l$$
$$0.024 = (30)c(1)$$
$$c = \frac{0.024}{30}$$
$$c = 0.0046\ g/L$$

Where:
- A = 0.024
- ε = 30
- l = 1

8.

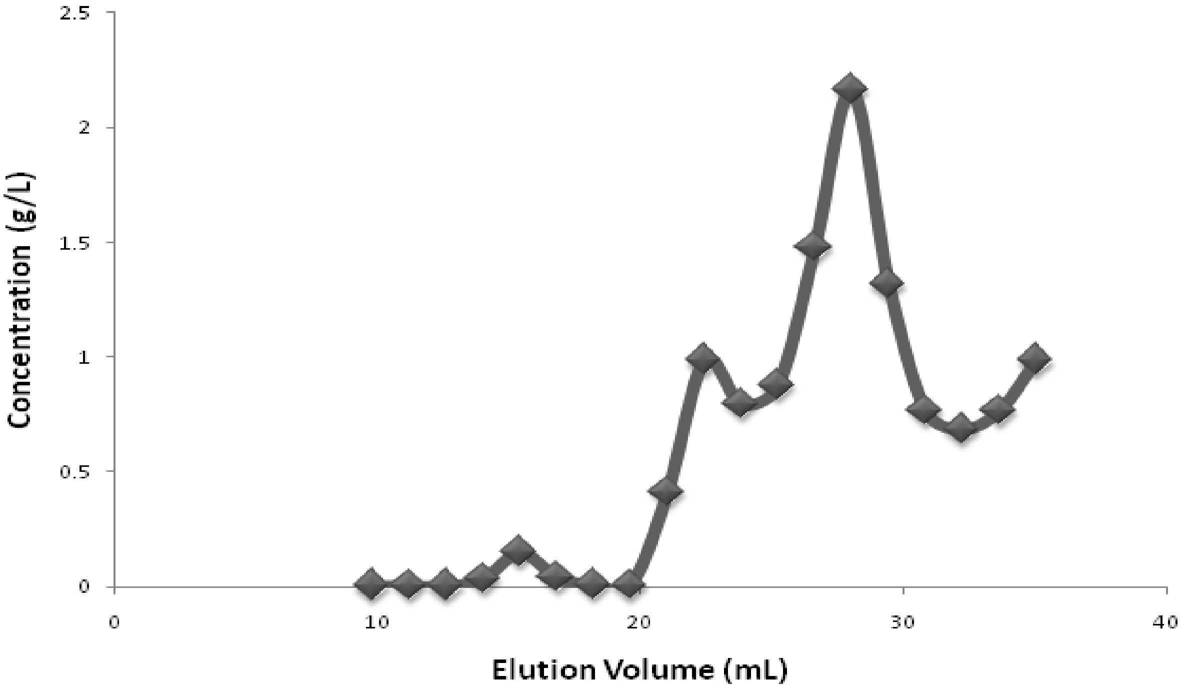

Figure #1: Concentration of glucose and starch plotted against the elution volume. The data was obtained by a gel filtration chromatography, which was then tested for various types of sugars and carbohydrates, the absorbances were then taken relative to water and the concentrations were then calculated.

When our results were graphed, it showed that we have three peaks. The peaks were located at 15.4 mL, 22.4 mL and 28.0 mL. From the obtained results, it is believed that the peak at 15.4 mL is for the starch while the 28.0 mL is for the glucose. This leaves the peak at 22.4 as an unwanted peak due to experimental error. The reason why only two peaks are desired is because of the fact that there were only two solutions that were eluted.

9.

Recovery Yield for Starch:

$$RC = \frac{\Sigma m_i}{\mathrm{m_{total}}} \times 100\%$$
$$RC = \frac{\Sigma m_i}{10\ \mathrm{mg}} \times 100\%$$
$$RC = \frac{10 - 0.357}{10\ \mathrm{mg}} \times 100\%$$
$$RC = 96.4\%$$

Where:

- RC = recovery yield
- m_i = mass of individual test tubes
- m_{tot} = total mass

Recovery Yield for Glucose:

$$RC = \frac{\Sigma m_i}{\mathrm{m_{total}}} \times 100\%$$
$$RC = \frac{\Sigma m_i}{10\ \mathrm{mg}} \times 100\%$$
$$RC = \frac{11.237}{500\ \mathrm{mg}} \times 100\%$$
$$RC = 2.24\%$$

Where:

- RC = recovery yield
- m_i = mass of individual test tubes
- m_{tot} = total mass

From the calculated recovery yields it was determined that there was a high recovery yield for the starch while there was a really low recovery yield for the glucose. The reason why there is a recovery yield that should approach 100% for glucose is because glucose is a smaller molecule that when given the opportunity

10.

First calculate V_t

$$v_t = \pi \left(\frac{d}{2}\right)^2 h$$
$$v_t = \pi \left(\frac{0.8}{2}\right)^2 (44)$$
$$v_t = 22.11\ mL$$

Where:

- d = 0.8 cm
- h = 44 cm

Second calculate K_{av}, for glucose

$$v_{av} = \frac{(v_e - v_o)}{(v_t - v_o)}$$
$$k_{av} = \frac{(28.0 - 15.0)}{(22.11 - 15.0)}$$
$$k_{av} = 1.82$$

Amylose:

$$v_{av} = \frac{(v_e - v_o)}{(v_t - v_o)}$$
$$k_{av} = \frac{(22.4 - 15.0)}{(22.11 - 15.0)}$$
$$k_{av} = 1.04$$

Amylopectin:

$$v_{av} = \frac{(v_e - v_o)}{(v_t - v_o)}$$
$$k_{av} = \frac{(15.4 - 15.0)}{(22.11 - 15.0)}$$
$$k_{av} = 0.0562$$

The carbohydrate with the lowest K_{av} would be the amylopectin. The reason for this is that molecularly amylopectin is the biggest molecule out of the three and also has side chain sugar molecules. For this reason, amylopectin is able to penetrate the pores the easiest. The sugar with the second least K_{av} would be the amylose. Once again this is also a very big molecule relative to glucose. The reason why amylose has a greater K_{av} than amylopectin is because amylose is a linear molecule that consists of no branching of sugars. The molecule with the greatest K_{av} would be the glucose. Glucose is a very small molecule that is not very effective at penetrating the pores.

11. If the column length were to be decreased by half, the k_{av} would not change. The reason why the k_{av} would not change is because there is no dependence of the column width on k_{av}. The only variable that k_{av} is dependent upon is the gel.
    On the other hand, if the column width were to be changed by a half, the v_e would also get decreased by a half. The reason why it would take less volume to eluate is because there is more sugar in each fraction due to the difference in column width.