

Lab BCH 2333

Section: C01 (Wednesday morning)

Lab 1: Carbohydrates

Puneet Labana

May 20th, 2020

5

**Sarah Khatib
300062670**

Purpose

This lab's goal is to properly carry out two experiments involving the separation of glucose and starch from a sample mixture. The first experiment is dialysis, a process that involving a dialysis bag filled with the sample mixture then submerged in water. The second experiment is gel filtration, a process that involves carefully eluting the sample mixture and then allowing the mixture to pass through a glass column filled with hydrated beads (Sephadex) to produce 15 fractions. After the completion of both experiments, a 3rd and 4th experiment is carried out to assay the results from dialysis and gel filtration to determine the extent the glucose and starch had been separated, dyes were also used in these stages to detect if the oxidase-peroxidase has been oxidized or its pH has changed.

Results & Discussion

R1

Table 1. Dialysis results for glucose and starch sample solution. 10 mL of starch-glucose solution was prepared and poured into a dialysis tube (1.6 cm radius, MM cut off: 12000 - 14000 Da). After clipping both ends of the dialysis tube, and ensuring no air was inside the dialysis tube, the tube was submerged in a beaker of 200 mL ultrapure water for approximately 45 minutes. After the dialysis, the internal and external solutions of the bag and beaker were assayed. Glucose absorption was measured at 420 nm wavelength and starch absorption was measured at 600 nm wavelength. Initial and final measurements for the glucose and starch solutions are depicted in Table 1.

t (min)	Glucose						Starch					
	A ₄₂₀ Internal solution	c ₁ (g/L)	m ₁ (mg)	A ₄₂₀ External solution	c ₂ (g/L)	m ₂ (mg)	A ₆₀₀ Internal solution	c ₁ (g/L)	m ₁ (mg)	A ₆₀₀ External solution	c ₂ (g/L)	m ₂ (mg)
0	0.306	57.6	564.48	-----	0	0	0.617	0.942	9.23	-----	0	0
45	1.527	59.88	586.82	0.147	1.153	230.59	0.550	2.33	22.85	-0.034	-9.04x10 ⁻⁴	-3.45 x 10 ⁻⁴

Explanation & Analysis

When the dialysis tube was submerged in the 200 mL water, osmosis occurred between the internal (starch-glucose concentration) and the external (pure water) solution. The pure water had the lowest solute concentration, therefore the concentration gradient travelled from the internal to the external solution.

Glucose is smaller than starch, it easily diffuses through the semipermeable membrane. Glucose oxidase and iodine also evidenced the concentration of glucose in the sample, with a yellow color indicating glucose, whereas a blue color indicating starch. The starch concentration after dialysis is negative, likely the result of human error while performing the dialysis or incorrect blank absorbance reading. However, this still demonstrates that starch is not capable of entering the external solution due to its bulky shape and has a low concentration in the exterior solution.

Calculations

1) Absorbance of internal solution A_{420} at $t=0$

$$A_{420} = [A_{\text{reading of tube w/ glucose-starch solution}}] - [A_{\text{of tube}_{\text{blank}}}]$$

$$A_{420} = 0.378 - 0.072$$

$$A_{420} = \mathbf{0.306}$$

2) Initial Glucose concentration at $t=0$

$$Ad = 0.306 \quad Ab = 0.255 \quad cb = 2.0 \text{ mg/mL}$$

$$\frac{Ab}{cb} = \frac{Ad}{\left(\frac{0.025}{6}\right) \cdot cd}$$

$$cd = (Ad) \cdot \left(\frac{cb}{Ab}\right) \cdot \left(\frac{0.6}{0.025}\right)$$

$$cd = (0.306) \cdot \left(\frac{2.0 \text{ mg/mL}}{0.255}\right) \cdot \left(\frac{0.6}{0.025}\right)$$

$$cd = 57.6$$

$$cd = \mathbf{57.6 \text{ g/L}}$$

3) Initial Glucose mass at $t=0$

$$c1 = 57.6 \text{ g/L} \quad V1 = 9.8 \text{ mL}$$

$$m1 = c1 \cdot V1$$

$$m1 = 57.6 \cdot 9.8$$

$$m1 = \mathbf{564.48 \text{ mg}}$$

4) Initial Starch concentration at $t=0$

$$Ac = 0.393 \quad Ad = 0.617 \quad cc = 0.1 \text{ mg/mL}$$

$$\frac{Ac}{cc} = \frac{Ad}{cd \cdot \left(\frac{0.1}{0.6}\right)}$$

$$cd = Ad \cdot \left(\frac{cc}{Ac}\right) \cdot \left(\frac{0.6}{0.1}\right)$$

$$cd = (0.617) \cdot \left(\frac{0.1}{0.393}\right) \cdot \left(\frac{0.6}{0.1}\right)$$

$$cd = 0.94198 \text{ mg/mL}$$

$$cd = \mathbf{0.942 \text{ g/L}}$$

5) *Initial Starch mass at t=0*

$$c1 = 0.49 \quad V1 = 9.8$$

$$m1 = c1 \cdot v1$$

$$m1 = 0.942 \cdot 9.8$$

$$m1 = \mathbf{9.23 \text{ mg}}$$

6) *Final Glucose concentration of internal solution at t=45*

$$Ab = 0.255 \quad Ae = 1.527 \quad cb = 2.0 \text{ mg/mL}$$

$$\frac{Ab}{cb} = \frac{Ae}{ce \cdot \left(\frac{0.6}{0.12}\right)}$$

$$ce = Ae \cdot \left(\frac{cb}{Ab}\right) \cdot \left(\frac{0.6}{0.12}\right)$$

$$ce = (1.527) \cdot \left(\frac{2.0}{0.255}\right) \cdot \left(\frac{0.6}{0.12}\right)$$

$$ce = 59.88235 \text{ mg/mL}$$

$$ce = \mathbf{59.88 \text{ g/L}}$$

7) *Final Glucose mass of internal solution at t=45*

$$c1 = 59.88 \quad V1 = 9.8$$

$$m1 = c1 \cdot v1$$

$$m1 = 59.88 \cdot 9.8$$

$$m1 = \mathbf{586.824 \text{ mg}}$$

8) *Final Glucose concentration of external solution at t=45*

$$Ab = 0.255 \quad Af = 0.147 \quad cb = 2.0 \text{ mg/mL}$$

$$\frac{Ab}{cb} = \frac{Af}{cf}$$

$$cf = Af \cdot \left(\frac{cb}{Ab}\right)$$

$$cf = (0.147) \cdot \left(\frac{2.0}{0.255}\right)$$

$$cf = 1.153 \text{ mg/mL}$$

$$cf = \mathbf{1.153 \text{ g/L}}$$

9) Final Glucose mass of external solution at $t=45$

$$c_2 = 1.153 \quad V_2 = 200 \text{ mL}$$

$$m_1 = c_2 \cdot V_2$$

$$m_1 = 1.153 \cdot 200$$

$$m_1 = \mathbf{230.59 \text{ mg}}$$

10) Final Starch concentration of internal solution at $t=45$

$$A_c = 0.393 \quad A_e = 1.527 \quad c_c = 0.1$$

$$\frac{A_c}{c_c} = \frac{A_e}{c_e \cdot \left(\frac{0.1}{0.6}\right)}$$

$$c_e = A_e \cdot \left(\frac{c_c}{A_c}\right) \cdot \left(\frac{0.6}{0.1}\right)$$

$$c_e = 1.527 \cdot \left(\frac{0.1}{0.393}\right) \cdot \left(\frac{0.6}{0.1}\right)$$

$$c_e = 2.33129 \text{ mg/mL}$$

$$c_d = \mathbf{2.33 \text{ g/L}}$$

11) Final Starch mass of internal solution at $t=45$

$$c_1 = 2.33 \quad V_1 = 9.8 \text{ mL}$$

$$m_1 = c_1 \cdot V_1$$

$$m_1 = 2.33 \cdot 9.8$$

$$m_1 = \mathbf{22.85 \text{ mg}}$$

12) Final Starch concentration of external solution at $t=45$

$$A_c = 0.393 \quad A_f = -0.034 \quad c_c = 0.1 \text{ mg/mL}$$

$$\frac{A_c}{c_c} = \frac{A_f}{c_f}$$

$$c_f = A_f \cdot \left(\frac{c_c}{A_c}\right)$$

$$c_f = (-0.034) \cdot \left(\frac{0.1}{0.393}\right)$$

$$c_f = -0.008651399 \text{ mg/mL}$$

$$c_f = \mathbf{-0.00865 \text{ g/L}}$$

13) Final Starch mass of external solution at $t=45$

$$c_2 = -0.00865 \quad V_2 = 200$$

$$m_2 = -0.00865 \cdot 200$$

$$m_2 = \mathbf{-1.730 \text{ mg}}$$

R2

Estimation of Glucose concentration at equilibrium

$$c_0 = 57.6 \quad V_1 = 9.8 \quad V_2 = 200$$

$$t = 62 \quad r = 1.6$$

$$c_{eq} = \frac{c_0 \cdot V_1}{V_1 + V_2}$$

$$c_{eq} = \frac{57.6 \cdot 9.8}{9.8 + 200}$$

$$= 2.69056 \text{ mg/mL}$$

$$c_{eq} = \mathbf{2.69 \text{ g/L}}$$

At equilibrium, the concentration of glucose is 2.69 g/L.

R3

Estimation of apparent diffusion coefficient D' of Glucose

$$c_0 = 57.6 \quad V_1 = 9.8 \quad V_2 = 200 \quad c_{eq} = 2.69 \quad c_1 = 59.88$$

$$t = 45 \quad r = 0.8$$

$$a = \frac{V_1}{V_2}$$

$$a = \frac{9.8}{200} = 0.049$$

$$(c_1 - c_{eq}) \cdot (1 + a) = c_0 e^{-Bt}$$

$$e^{-Bt} = \frac{(c_1 - c_{eq}) \cdot (1 + a)}{c_0}$$

$$e^{-Bt} = \frac{(59.88 \text{ mg/mL} - 2.69 \text{ mg/mL}) \cdot (1 + 0.049)}{57.6}$$

$$e^{-Bt} = 1.04153$$

$$-Bt = \ln(1.04153)$$

$$-Bt = 0.040693 = x$$

$$B = -0.000904307 \div (45)$$

$$B = -9.04 \times 10^{-4} \text{ min}^{-1}$$

$$B = \frac{2(1 + a) \cdot D'}{r}$$

$$D' = \frac{B \cdot r}{2(1 + 0.049)}$$

$$D' = \frac{(-9.04 \times 10^{-4} \text{ min}^{-1}) \cdot (0.8 \text{ cm})}{2(1 + 0.049)}$$

$$D' = -3.45 \times 10^{-4} \text{ cm/min}$$

Across the dialysis membrane, the diffusion coefficient of glucose will be -3.45×10^{-4} cm/min.

R4

Table 2. Fraction elution volume results for glucose and starch. Sephadex (MW cut off: 5000 Da) and distilled water was poured into a glass column (1.0 cm x 50 cm). By positioning the glass column vertically, the Sephadex in distilled water ensured no air bubbles were trapped in the glass column ensuring a smooth transition from column to sample syringe. The average fraction volume was given as 3.7 mL. The approximate time to collect all 15 fraction was 40 minutes. Once gel filtration process was complete, all 15 fractions were assayed. Starch absorbance was measured at 600 nm wavelength, and glucose absorbance was measured at 420 nm wavelength. Table 2 depicts elution volumes, absorbances, masses and concentration for both glucose and starch standards.

Fraction		Glucose			Starch		
#	Elution Vol. (mL)	A ₄₂₀	c (g/L)	Mass (mg)	A ₆₀₀	c (g/L)	Mass (mg)
1	3.7	0.021	0.137	0.507	0.011	0.00178	0.00659
2	7.4	0.021	0.137	0.507	0.031	0.00502	0.0186
3	11.1	0.025	0.163	0.603	0.026	0.00421	0.0156
4	14.8	0.029	0.189	0.699	0.027	0.00438	0.0162
5	18.5	0.039	0.255	0.944	0.246	0.0398	0.147
6	22.2	0.094	0.614	2.272	0.142	0.023	0.0851
7	25.8	0.394	2.575	9.528	0.045	0.00729	0.0269
8	29.6	0.305	1.993	7.374	0.015	0.00243	0.00899
9	33.3	0.105	0.686	2.538	0.025	0.00405	0.0149
10	37	0.056	0.366	1.354	0.024	0.00389	0.0144
11	40.7	0.027	0.176	0.651	0.015	0.00243	0.00899
12	44.4	0.030	0.196	0.725	0.010	0.00162	0.00599

13	48.1	0.037	0.242	0.895	0.030	0.00486	0.0179
14	51.8	0.026	0.169	0.625	0.025	0.00405	0.0149
15	55.5	0.036	0.235	0.869	0.015	0.00243	0.00899

1) Fraction Elution Volume for Fraction 6

$$F\# = 6 \quad V_{avg} = 3.7$$

$$EV = F\# \times V_{avg}$$

$$EV = 6 \times 3.7$$

$$EV = 22.2$$

2) Glucose Concentration for Fraction 6

$$Ab = 0.306 \quad Ag6 = 0.094 \quad cb = 2.0 \text{ mg/mL}$$

$$\frac{Ab}{cb} = \frac{Ag6}{cg6}$$

$$cg6 = Ag6 \cdot \left(\frac{cb}{Ab}\right)$$

$$cg6 = (0.094) \cdot \left(\frac{2.0}{0.306}\right)$$

$$cg6 = 0.614379 \text{ mg/mL}$$

$$cg6 = 0.614 \text{ g/L}$$

3) Glucose mass for Fraction 6

$$c = 0.614 \text{ g/L} \quad V_{avg} = 3.7$$

$$m = c \cdot V_{avg}$$

$$m = (0.614) \cdot (3.7)$$

$$m = 2.272 \text{ mg}$$

4) Starch Concentration for Fraction 6

$$Ac = 0.617 \quad Ag6 = 0.142 \quad cc = 0.1$$

$$\frac{Ac}{cc} = \frac{Ag6}{cg6}$$

$$cg6 = Ag6 \cdot \left(\frac{cc}{Ac}\right)$$

$$cg6 = 0.142 \cdot \left(\frac{0.1}{0.617}\right)$$

$$cg6 = 0.023 \text{ g/L}$$

5) Starch mass for Fraction 6

$$c = 0.023 \quad V_{avg} = 3.7$$

$$m = c \cdot V_{avg}$$

$$m = 0.023 \cdot 3.7$$

$$m = 0.0851 \text{ mg}$$

R5

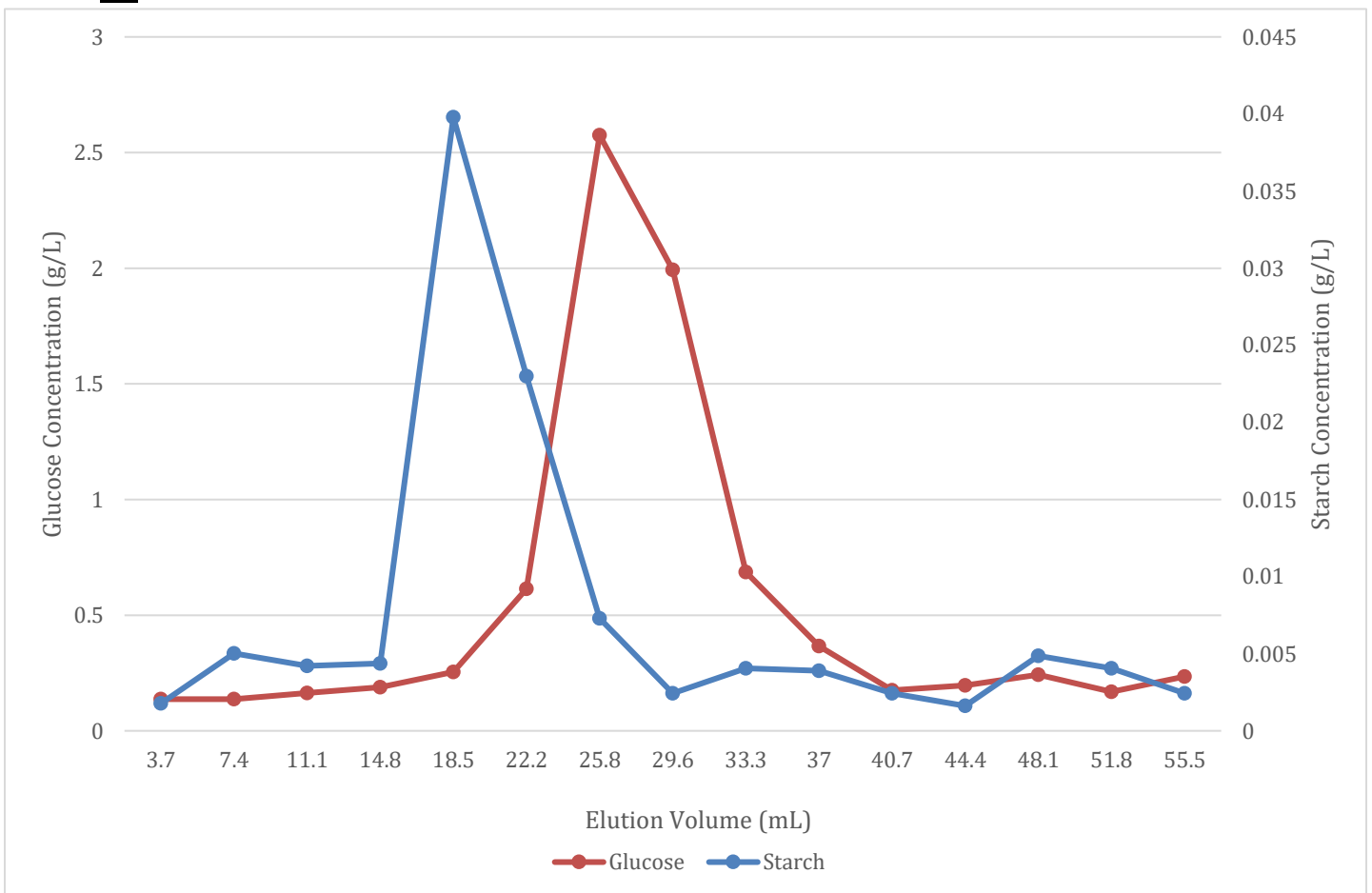


Figure 1. Concentration of Starch and Glucose for Fraction Elution Volumes. The graph demonstrates a trend in starch and glucose concentrations for the 15 fraction volumes that underwent gel infiltration. On the y-axes represent the glucose and starch concentrations (g/L) are depicted, and the x-axis represents the elution volume (mL) for the 15 fractions. As already stated for Table 2, Sephadex (MW cut off: 5000 Da) and distilled water were poured into a glass column (1.0 cm x 50 cm) for the gel filtration chromatography. After accumulating all 15 fractions, the absorbance readings were collected at 420 nm for glucose and 600 nm for starch. Using the absorbance readings, the concentrations and mass of glucose and starch can be calculated.

Explanation and Analysis

Two prominent peaks were expected and then observed for each trend, the glucose peak is broad and the starch peak is sharp. For glucose, the peak reaches 2.575 g/L at elution 25.8 mL, demonstrating that glucose in the glucose-starch mixture was reduced. Glucose's small size allows it to intercept the hydrated beads, in comparison, starch is a larger molecule, and would only pass through the column because it cannot be intercepted by the beads. Due to this, starch accumulated faster than glucose and the starch peak (0.0398 g/L at elution 18.5 mL) appears first before the glucose peak, showing a significant gap between starch and glucose accumulation in all 15 fractions. The void volume is 15 mL, in comparison to the peak starch concentration (18.5 mL) that is close to what was expected for the elution volume. The peak starch volume is less than the peak glucose volume due to the necessary precaution of ensuring no air bubbles enters the columns.

R6

1) Calculation of V_t

$$d = 1.0 \text{ cm} \quad h = 44.9 \text{ cm} \quad V(\text{starch}) = 18.5 \quad V(\text{glucose}) = 25.8 \quad V_0 = 15 \text{ mL}$$

$$V_t = \pi \cdot \left(\frac{d}{2}\right)^2 \cdot h$$

$$V_t = (3.14) \cdot \left(\frac{1.0}{2}\right)^2 \cdot (44.9)$$

$$V_t = 70.49 \text{ cm}^3$$

$$V_t = 70.49 \text{ mL}$$

2) Average distribution coefficient (K_{av}) of Glucose

$$K_{av} = \frac{v_e - v_0}{v_t - v_0}$$

$$K_{av} = \frac{25.8 - 15}{70.49 - 15}$$

$$K_{av} = \mathbf{0.195}$$

3) Average distribution coefficient (K_{av}) of Starch

$$d = 1 \text{ cm} \quad h = 44.9 \text{ cm} \quad V(\text{starch}) = 18.5 \quad V(\text{glucose}) = 25.8 \quad V_0 = 15 \text{ mL}$$

$$V_t = 39.25$$

$$K_{av} = \frac{v_e - v_0}{v_t - v_0}$$

$$K_{av} = \frac{18.5 - 15}{70.49 - 15}$$

$$K_{av} = \mathbf{0.0631}$$

The average distribution coefficient for Glucose is 0.195 on a range of $0 < K_{av} < 1$ (with small molecules being closer to 0). This interval is smaller than anticipated, but it demonstrates that it is closer to 1 and therefore a small molecule capable of diffusing through a semipermeable membrane.

The average distribution coefficient for Starch is 0.0361, this result is close to 0 and therefore indicates that it is a large molecule incapable of diffusing through a semipermeable membrane.

R7

1) *Recovery yield (%) of column for Glucose*

$$cI = 57.6 \quad Vi = 0.5 \text{ mL}$$

$$m = cI \cdot Vi$$

$$m = 57.6 \cdot 0.5$$

$$m = 28.8 \text{ mg}$$

m(gel filtration)

$$= 0.507 + 0.507 + 0.603 + 0.699 + 0.944 + 2.272 + 9.528 + 7.374 + 2.538 \\ + 1.354 + 0.651 + 0.725 + 0.895 + 0.625 + 0.869$$

$$m = 30.091 \text{ mg}$$

$$\% \text{ Recovery Yield} = \frac{30.091}{28.8} \cdot 100$$

$$\% \text{ Recovery Yield} = \mathbf{104.48\%}$$

2) *Recovery yield (%) of column for Starch*

$$cI = 0.942 \quad Vi = 0.5 \text{ mL}$$

$$m = cI \cdot Vi$$

$$m = 0.49 \cdot 0.5$$

$$m = 0.471 \text{ mg}$$

$$m(\text{gel filtration}) = 0.00659 + 0.0186 + 0.0156 + 0.0162 + 0.147 + 0.0851 + 0.0269 + \\ 0.00899 + 0.0149 + 0.0144 + 0.00899 + 0.00599 + 0.0179 + 0.0149 + 0.00899 = 0.41105 \\ \text{mg}$$

$$\% \text{ Recovery Yield} = \frac{0.41105}{0.471} \cdot 100$$

$$\% \text{ Recovery Yield} = \mathbf{87.27\%}$$

Conclusions

Recovery yield for glucose is 104.48%, which indicates that more than 100% of the glucose molecules were recovered from the gel filtration process. Likely, this value is a result of human error during the gel filtration stage, the stop valve was likely not closed properly and more glucose was intercepted by the beads than what was necessary. In Figure 1, a second, smaller peak is observed after the large peak, which likely represents the extra glucose that fell down the column slowly.

Recovery yield for starch is 87.27%, that indicates less 20% of starch was lost during the filtration process. Though most of the starch molecules were recovered, a possible reason as to why starch molecules were lost is likely that some starch were stuck or was clinging to the beads as it passed down the column. In Figure 1, the starch trend shows minor fluctuations after the peak was reached, this fluctuation is likely due to the randomness of the starch intercepting the hydrated beads to reach the bottom of the column as it accumulated in the first few fractions.

R8

Yes.