Gel Chromatography out-of-lab Assignment

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Introduction

According to the lab protocol written by the coordinators Dr. D'Souza and Dr. Julien Gibon (2020), the main purpose of the experiment was to use gel chromatography in order to efficiently separate and characterize five different components initially within one solution. The coloured components in the solution were the proteins cytochrome C (MW 12,384) and FITC-bovine albumin (MW 66,000) as well as the markers blue dextran (MW 2,000,000) and vitamin B_{12} (MW 1,257). The previously mentioned components were to be characterized through spectrophotometry, whereas the colourless component, SBTI (MW ~20,000), was to be characterized through the post-laboratory calculations.

The results to be reported in the next couple of sections are the plots, calculations and conclusions made as the post-laboratory assignment for this experiment.

Results

Having performed the chromatography experiment, it was observed that the colour of the eluted solution slowly changed colour, first turning blue, then yellow, orange and pink; which led to the conclusion that the sequence of elusion of components was respectively Blue Dextran, FITC Albumin, Cytochrome C and Vitamin B_{12}, according to their characteristic colours as mentioned in the lab protocol (D'Souza & Gibon, 2020).

Spectrophotometry was the assigned method of assay for this experiment and it was done in four different wavelengths in order to confirm the elution sequence of the sample components as well as their distribution among the fractions. The analysis of the data obtained from the assay can be observed in the elution profile demonstrated below in Figure 1:

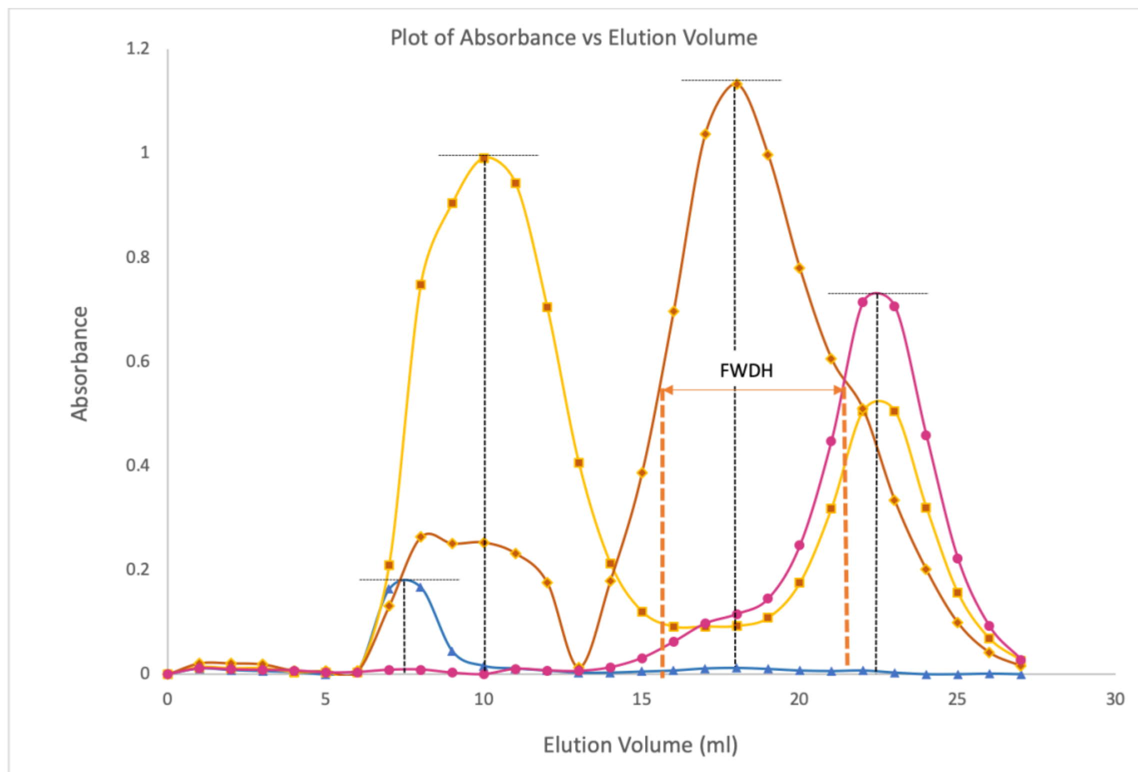

Figure 1. Scatter Plot of Absorbance Versus (AU) Elution Volume (ml) with relation to wavelength. The blue, yellow, orange and pink lines represent the wavelengths of 625nm, 550nm, 500nm and 410nm as well as Blue Dextran, FITC Albumin, Cytochrome C and Vitamin B_{12} respectively. The maximum amount of each of the coloured components is indicated by dashed lines coming vertically from the x-axis.

From the plotted elution profile, the Ve values for each of the coloured components were determined in accordance to the peak estimation method in the lab protocol (D'Souza & Gibon, 2020), which stated that the Ve values were equal to the volume "corresponding to the front of the peak, measured at full width of the peak at half of the maximum peak height, or FWDH", given 1.0 mL fractions. The method was applied to each one of the coloured sample components as exemplified in the curve for Cytochrome C in Figure 1 and it was then determined that the Ve for Blue Dextran, FITC Albumin, Cytochrome C and Vitamin B_{12} was 7mL, 8mL, 16mL and 21mL respectively.

Having determined the Ve for each one of the components, the distribution coefficient (K) and Log MW was the next one to be calculated. In order to calculate K, the values of total volume of the column (Vt), void volume (Vo) and static volume (Vs) were still to be determined.

The V_t was calculated through plugging in the radius and the height of the column into the formula for volume of a cylinder, as showed by Equation 1:

$$(Eq. 1)\quad V_C = \pi\, x\, r^2\, x\, h$$

Where V_c corresponds to cylinder volume, r corresponds to radius of the column and h corresponds to height of the column. This result was then confirmed by checking the elution volume for the smallest sample component, vitamin B12, since its complete inclusion within the column beads make it a marker of total volume. In contrast, a complete exclusion of a sample component from the column beads make it a good marker for void volume (V_o) by as mentioned in the lab protocol (D'Souza & Gibon, 2020). Due to these reasons, V_o was determined as 7mL, the same value as the elution volume for the biggest sample component, Blue Dextran.

Lastly, the static volume (Vs) was determined through plugging in the recently obtained values in Equation 2 shown below, which was provided by the lab protocol (D'Souza & Gibon, 2020).

$$(Eq. 2)\ V_T = V_O + V_S$$

Proceeding these calculations, the distribution coefficient (K) was determined for each one of the components by plugging the obtained values into Equation 3 from the lab protocol (D'Souza & Gibon, 2020) as shown below:

$$(Eq. 3)\ V_e = V_O + KV_S$$

The results for elution volume, distribution coefficient and Log MW for each one of the coloured components were tabulated and are demonstrated by Table 1 as follows:

Table 1

Elution Volume, Distribution Coefficient and log MW for each sample component

Sample Component	Elution Volume (ml)	Distribution Coefficient (k)	Log MW
Blue Dextran	7	0	6.30103
FITC Albumin	8	0.069252078	4.81954394
Cytochrome C	16	0.623268698	4.09286094
Vitamin B12	21	1	3.09933528

The values showed in Table 1 were then used to plot a molecular weight calibration curve of distribution coefficient K vs. LogMW in order to characterize the colourless compound present in the solution. The calibration curve was plotted as shown by Figure 2 below:

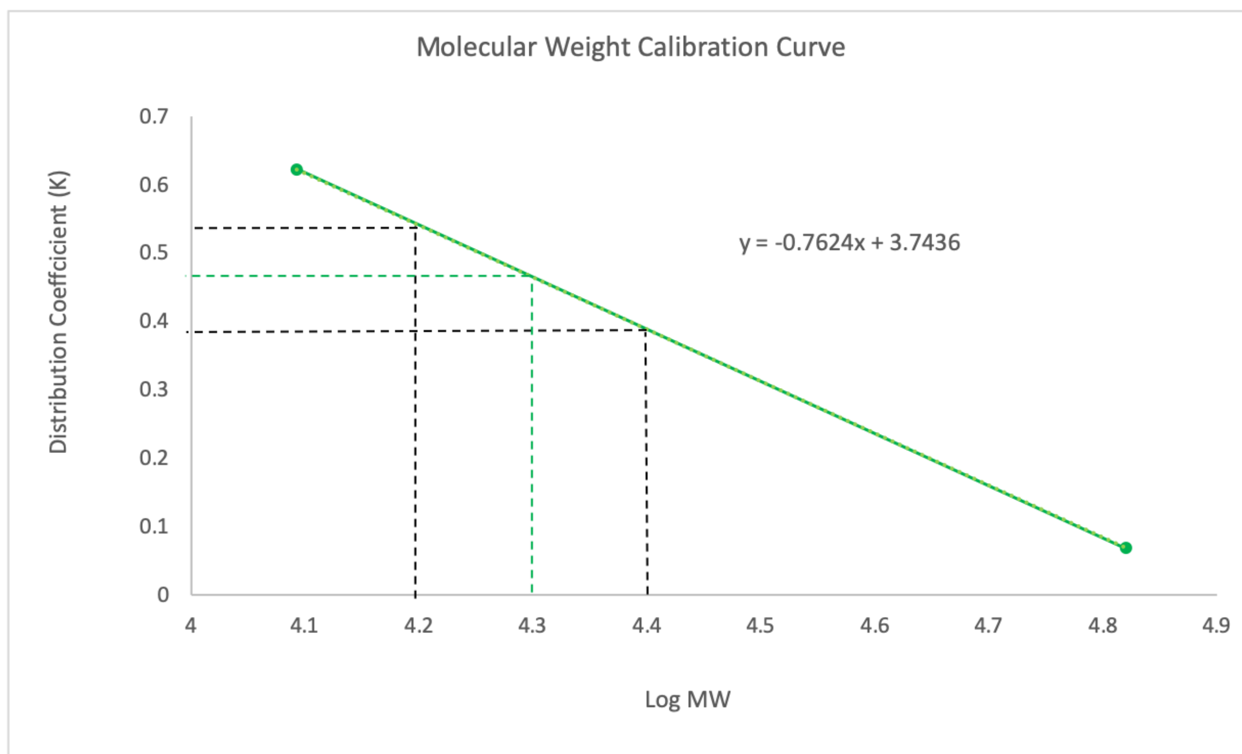

Figure 1. Scatter Plot representing a calibration curve between distribution coefficient and Log MW. The dashed lines on the standard curve are interpolation lines done in order to obtain K for the colourless component. The interpolation line highlighted as green was the one that determined the distribution coefficient for SBTI.

The plot for K vs. Log MW resulted in a straight line because the components either completely included or excluded from the column were not added as data points, once they represent the fractionation limits of the column, as asserted by Dr. D'Souza and Dr. Julien Gibon in the lab protocol (2020).

Following the plotting of the calibration curve, a trendline was added to the data series in order to obtain a linear equation. Shown below as Equation 4:

$$(Eq. 4) \quad y = -0.7624x + 37436$$

Where y represents the distribution coefficient values located in the y-axis, and x represents the Log MW values located in the x-axis. The value of distribution coefficient K for the colourless compound in the original solution was then determined by using the compound's estimated molecular weight of ~20,000 as according to the lab protocol (D'Souza & Gibon,

2020), transforming it into Log MW, plugging it into Equation 4 as a value of x and solving it for y.

The value for the distribution coefficient obtained from Equation 4 was confirmed by drawing interpolation lines on the plot as showed by Figure 2. This value allowed for determination of elution volume (Ve) for the colourless compound through Equation 3, which resulted in an Elution volume between 13 and 14mL; thus leading to the conclusion that the fraction range number with the highest quantity of SBTI is most likely 13 – 14 mL.

Conclusion

As mentioned in the introduction section of this report, according to the lab protocol (D'Souza & Gibon, 2020), the goal of this experiment was to use gel chromatography in order to efficiently separate and characterize five different components initially within one solution; Four of which were coloured, and therefore could be characterized by spectrophotometry, and one of which was colourless and had to be characterized through calculation and analysis of absorbance data. By these paraphrased objectives, it can be concluded that the experiment was completed successfully, having obtained all the data accurately enough to fully characterize the elution volume of the colourless compound as well as having gotten all the coloured components to elute from the column in their proper fraction range.

References

D'Souza, B., & Gibon, J. (2020). Lab 2: Protein Separation by Gel Filtration. Kelowna, BC: University of British Columbia.

Appendix A: Raw Tabulated Data

Table 1

Absorbance in Relation to Fraction Number and Wavelength

Fraction Number	Absorbance			
	625nm	550nm	500nm	410nm
0	0	0	0	0
1	0.011	0.011	0.013	0.021
2	0.008	0.009	0.011	0.021
3	0.006	0.008	0.011	0.019
4	0.004	0.007	0.004	0.007
5	0	0.003	0.004	0.006
6	0.006	0.004	0.004	0.006
7	0.164	0.0082	0.21	0.131
8	0.167	0.0087	0.748	0.263
9	0.044	0.0026	0.904	0.251
10	0.016	0	0.991	0.253
11	0.011	0.01	0.942	0.232
12	0.007	0.007	0.704	0.176
13	0.003	0.006	0.406	0.0131
14	0.003	0.013	0.213	0.179
15	0.005	0.031	0.12	0.386
16	0.007	0.062	0.091	0.696
17	0.011	0.097	0.091	1.036
18	0.012	0.115	0.092	1.133
19	0.01	0.145	0.108	0.996
20	0.007	0.247	0.175	0.779
21	0.006	0.448	0.318	0.606
22	0.007	0.714	0.505	0.509
23	0.003	0.706	0.505	0.334
24	0	0.459	0.32	0.201
25	0	0.222	0.156	0.099
26	0.001	0.092	0.068	0.042
27	0	0.028	0.026	0.016

Appendix B: Sample Calculations

Example Calculation for Total Volume (Vt):

$$V_C = \pi \, x \, r^2 \, x \, h$$

$$V_t = V_C = \pi \, x \, (0.5)^2 \, x \, 27.30$$

$$V_t = 21.44ml$$

Example Calculation for Static Volume (Vs):

$$V_T = V_O + V_S$$

$$21.44mL = 7mL + V_S$$

$$V_S = 14.44mL$$

Example Calculation for Elution Volume (Ve):
In this sample, the Elution volume value used was the one determined for the marker Cytochrome C.

$$V_e = V_O + KV_S$$

$$16 = 7 + K \, x \, (14.44)$$

$$K = 0.623268698$$

Example Calculation for Determining the K Value of the Colourless Compound:

$$y = -0.7624x + 37436$$

$$y = -0.7624(\log(20{,}000)) + 37436$$

$$y = K = 0.4644$$

Example Calculation for Determining the Elution Volume of the Colourless Compound:

$$V_e = V_O + KV_S$$

$$V_e = 7mL + (0.4644) \, x \, (14.44ml)$$

$$V_e = 13.707mL$$