

✓ Instructor Signature _____

NAME _____

SECTION _____

SIZE EXCLUSION CHROMATOGRAPHY
Worksheet #5

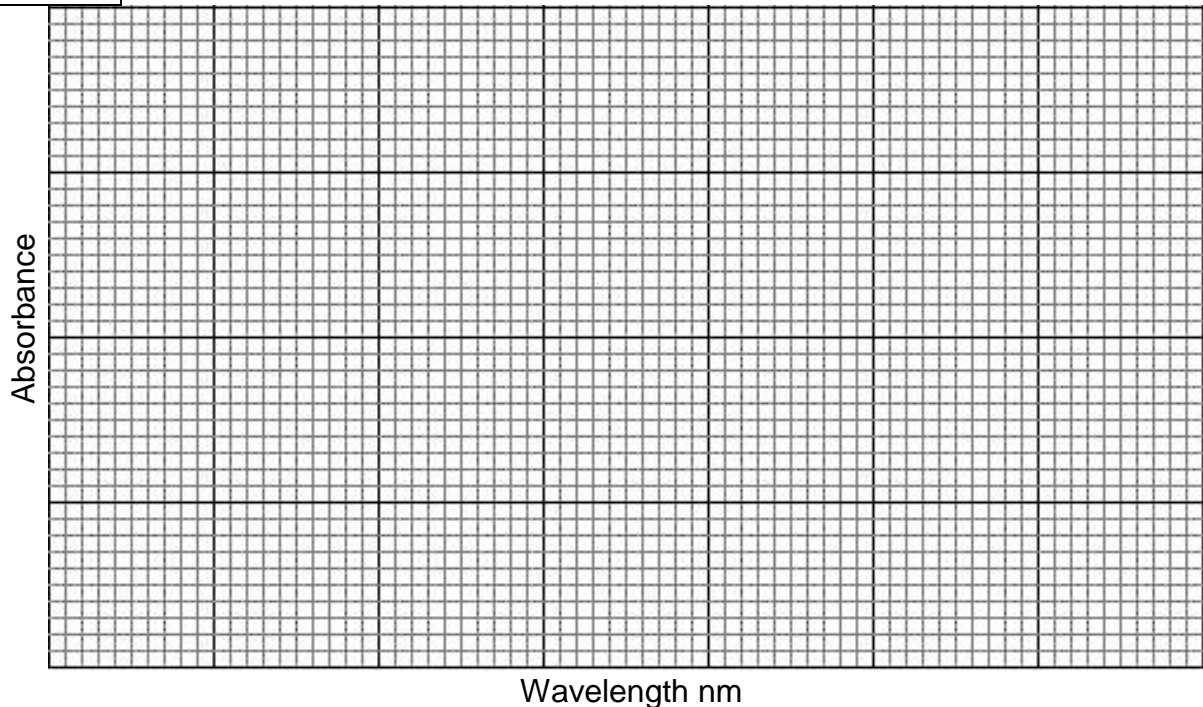
Lab Partners

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PART 2: DETERMINATION OF THE ABSORPTION SPECTRUM OF VITAMIN B12:

- Record the Absorbance for the wavelengths from 380nm to 680nm. *Plot your results below.*

| | | | | | | | | | |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 380nm | 390nm | 400nm | 410nm | 420nm | 430nm | 440nm | 450nm | 460nm | 470nm |
| | | | | | | | | | |
| 480nm | 490nm | 500nm | 510nm | 520nm | 530nm | 540nm | 550nm | 560nm | 570nm |
| | | | | | | | | | |
| 580nm | 590nm | 600nm | 610nm | 620nm | 630nm | 640nm | 650nm | 660nm | 670nm |
| | | | | | | | | | |
| 680nm | | | | | | | | | |
| | | | | | | | | | |



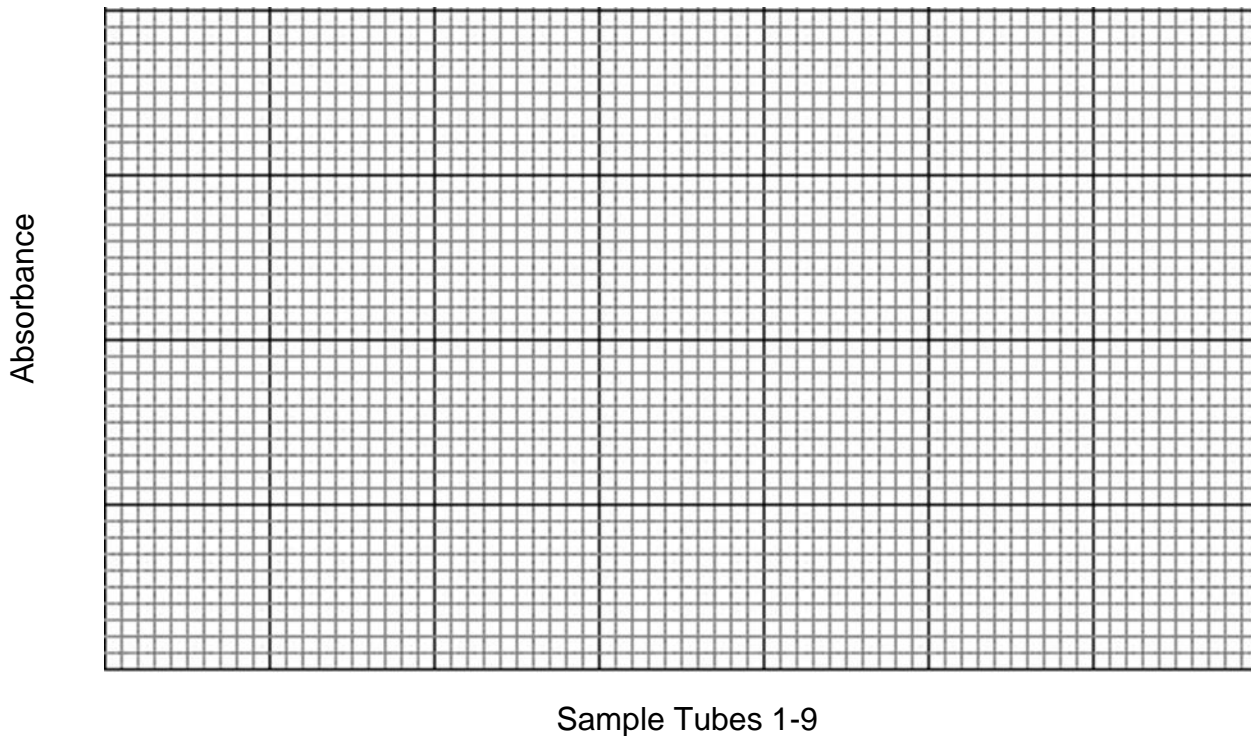
- What is the *A_{max}* vitamin B12? _____

PART 3: SPECTROPHOTOMETRIC DETECTION OF VITAMIN B12:

1. Record the Absorbance data for each of the 9 tubes you collected 5 drops of column runoff.

| | | | |
|----------------|--|-----------------|--|
| TUBE 2) | | TUBE 7) | |
| TUBE 3) | | TUBE 8) | |
| TUBE 4) | | TUBE 9) | |
| TUBE 5) | | TUBE 10) | |
| TUBE 6) | | | |

2. What wavelength did you use to screen for the presence of vitamin B12? _____
3. Plot your absorbance data from Part 3 on the graph below.



4. Which tubes did you detect increased absorbance? _____
5. What should an increase in absorbance indicate?

6. What observations can you make regarding the color of the column eluent? Were they all the same? Why would they be different?

7. Can you tell if your column separated vitamin B12 and hemoglobin? _____

What are some ways you would be able to tell?

ANALYSIS AND DISCUSSION:

1. Considering the design of this experiment, how should you be able to tell if vitamin B12 is present in some of your sample collection tubes?

2. Considering the design of this experiment, how should you be able to tell if hemoglobin is present in some of your sample collection tubes?

3. In the space below, draw a model of a column and describe how its design enables it to separate molecules.

4. What is an exclusion limit? What is the exclusion limit of the columns we are using today?

5. In today's activity, which molecules will be fractionated and which will be excluded?

6. Why is it necessary to use buffer throughout this activity?

7. What is the most likely assumption if you were not able to detect the presence of vitamin B12 in any of your collection tubes (following the use of a size exclusion column)?

If you could not detect vitamin B12, /what would your next step be in trying to determine where it is? *Explain your response.*