✓ Instructor Signature _____ **NAME** _____

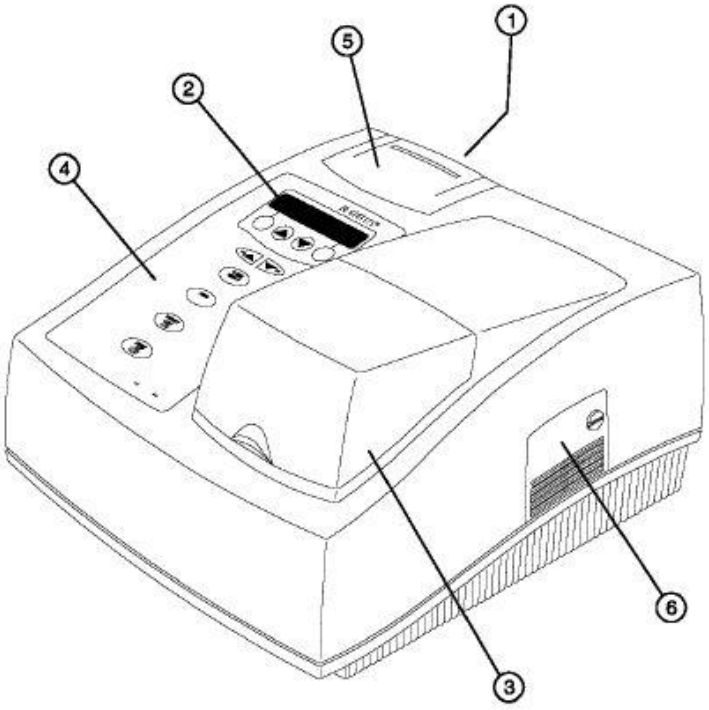
SECTION _____

SPECTROPHOTOMETRY

Worksheet #4

Lab Partners

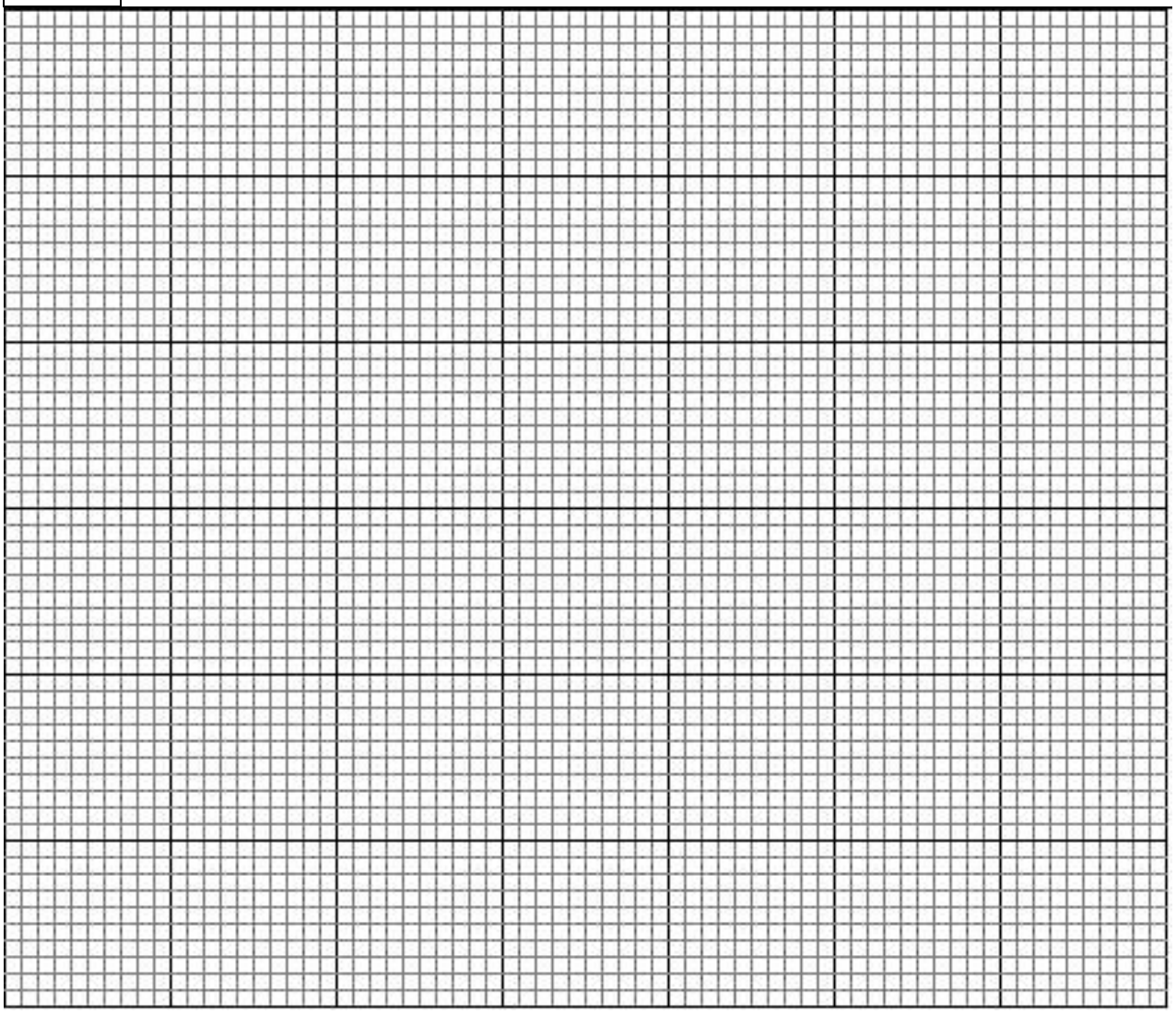
PART 1: DETERMINATION OF THE A*max* OF BROMOPHENOL BLUE:



1. Prior to determining the actual Amax of bromophenol blue, take a moment to estimate the approximate wavelength that you might expect this solution to possess.

2. Record the Absorbance for the range assigned to you group, and then transcribe the data from the other teams to complete the chart. *Plot results on graph below*.

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	690nm
700nm									



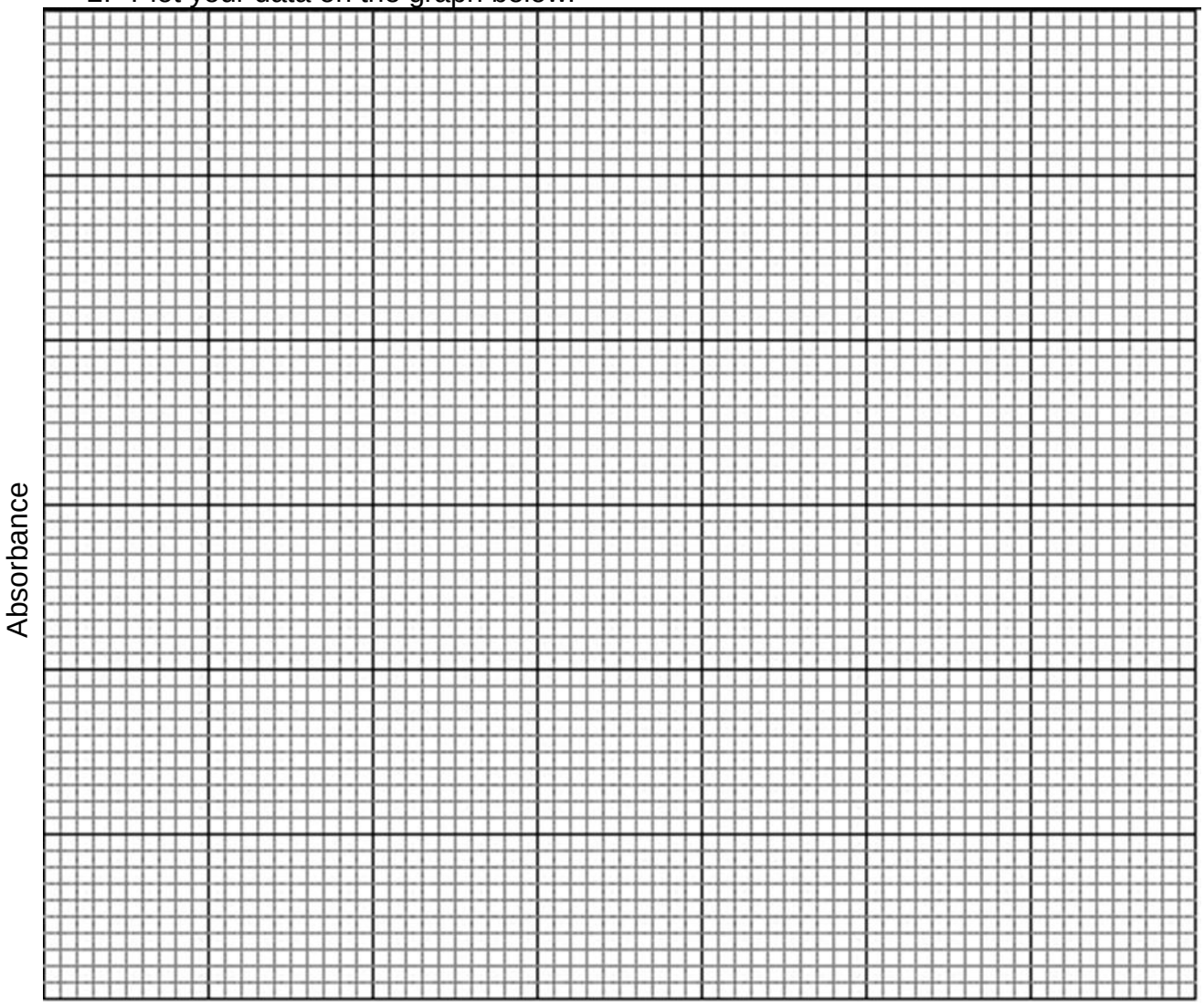
3. What is the A*max* for bromophenol blue? _____

PART 2: THE EFFECT OF CONCENTRATION ON ABSORBANCE: (*serial dilutions*)

1. Record the Absorbance and Concentrations for the serial dilutions of bromophenol blue in each tube

	Tube 1	**Tube 2**	**Tube 3**	**Tube 4**
Absorbance				
Dilution Factor				
Concentration				

2. Plot your data on the graph below.



3. What is the Absorbance of the unknown solution from section 2B? _____

4. What is the Absorbance of a 1:2 dilution of the unknown from 2B? _____

5. What is the Absorbance of a 1:3 dilution of the unknown from 2B? _____

6. Using the graph you created (plotting Absorbance vs Concentration) to determine the concentration of the unknown.

Unknown	No Dilution	1:2 Dilution	1:3 Dilution	Other
Absorbance				
Dilution Factor				
Estimated Concentration				

Concentration of unknown solution of Bromophenol Blue _____

ANALYSIS AND DISCUSSION:

1. If you make a 1:3 dilution of a 4M solution, what would the resulting concentration be?

   Reviewing your results, if you made a 1:3 dilution of a solution, can you just divide the Absorbance of your original sample by 3 to determine the absorbance? Please justify your response.

2. What is the relationship between absorbance and concentration?

3. Explain what the purpose of a 'blank' is in a spectroscopy procedure

   If you are determining the absorbance of an unknown substance that is dissolved in chloroform, what would you use to blank the spectrophotometer?

PRACTICE CALCULATIONS:

1. Describe how you would create a series of dilutions with a dilution factor of 4. Assume you are starting with a 200µl sample and you will make a series of 5 dilutions.

   Dilution 1: _____ µl of sample into _____ µl of diluent Total Vol _____
   Dilution 2: _____ µl of dilution 1 into _____ µl of diluent Total Vol _____
   Dilution 3: _____ µl of dilution 2 into _____ µl of diluent Total Vol _____
   Dilution 4: _____ µl of dilution 3 into _____ µl of diluent Total Vol _____
   Dilution 5: _____ µl of dilution 4 into _____ µl of diluent Total Vol _____

2. Describe how to make 20mls of a 0.20M solution of NaCl from a 2M stock solution. *Show your work!*

3. Describe how to make 300mls of a 0.05M Solution of NaOH from a 2M stock solution.

NO CONTENT