Spectrophotometric Analysis

UM-SJTU JI

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OBJECTIVES

- Practice calculating and performing dilutions of solutions.
- Construct and utilize an absorbance and calibration curve.
- Determine the concentration of phosphate in a water sample by spectrophotometric analysis:
  a. Using projection from the standard curve.
  b. Using slope of the standard curve then Beer's law [A = slope x c) & A = - Log(%T/100)]

BACKGROUND

- Spectrophotometric Analysis and the Determination of Phosphate
- The Absorbance Spectrum
- The Calibration Curve

Chromogenic Reaction of Phosphate

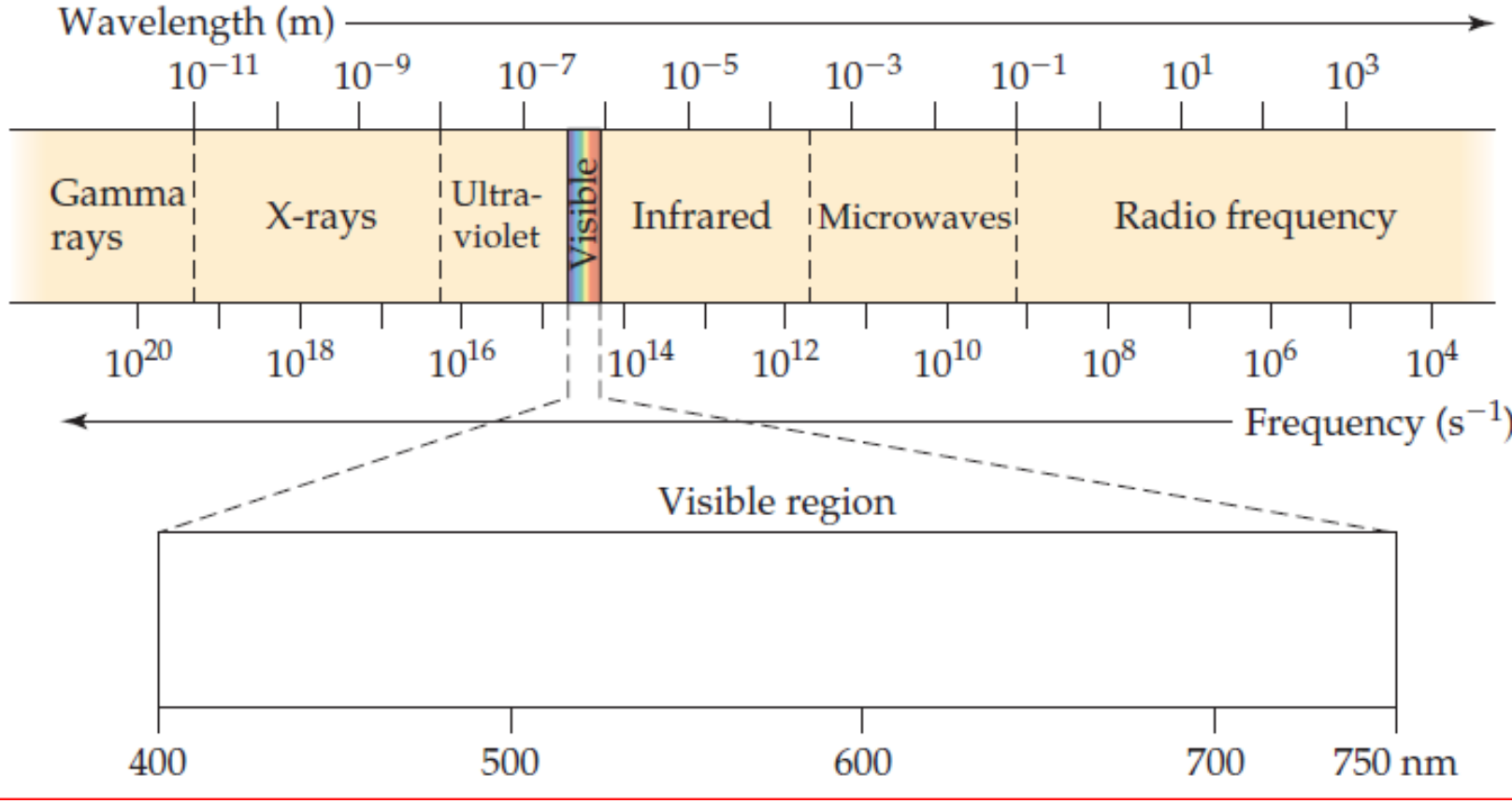
$$NH_4VO_3 + MoO_4^{2-} + PO_4^{3-}$$

$$\rightarrow \quad (NH_4)_3PO_4 \cdot NH_4VO_3 \cdot 16MoO_3$$

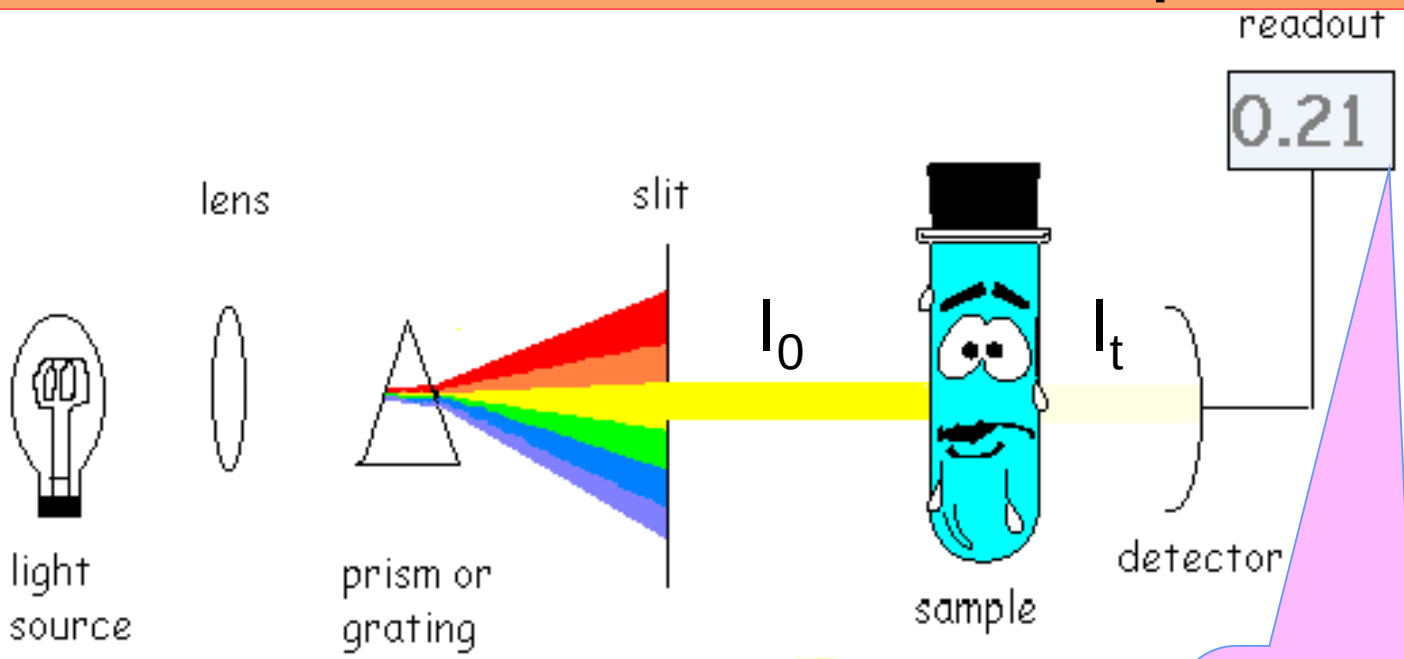
Chromogenic Reagent, **AV** solution

LIGHT SPECTRUM

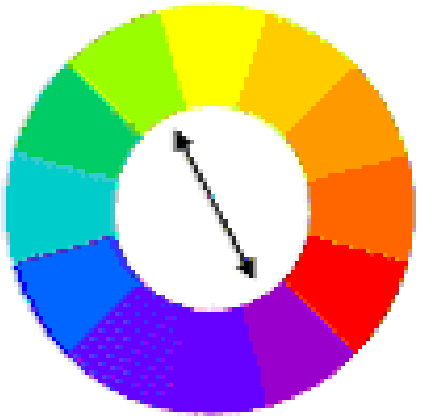
How do the wavelength and frequency of an X-ray compare with those of the red light from a neon sign?



Spectrophotometric Analysis and the Determination of Phosphate



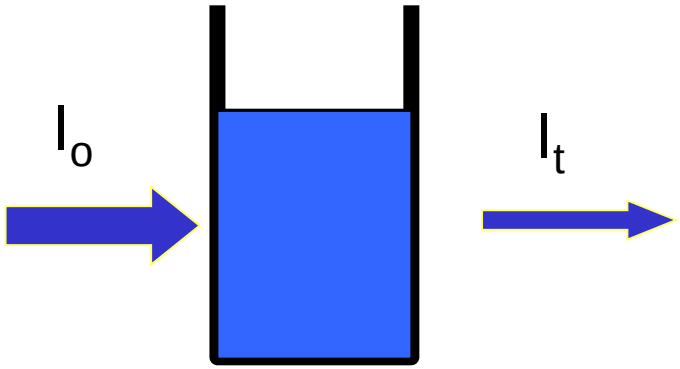
Complimentary color



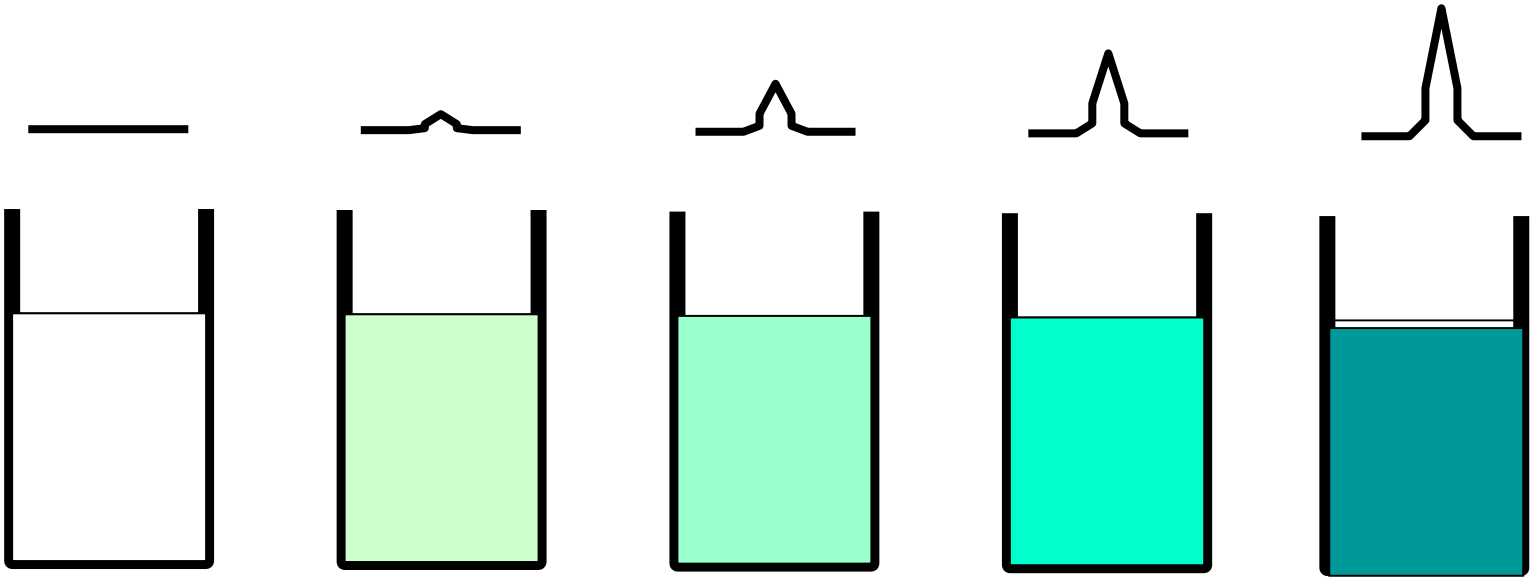
Color absorbed or transmitted?

Lambert—Beer Law

- I_o = intensity of light through blank
- I_t = intensity of light through sample
- Absorption = I_o - I_t
- Transmittance = I_t/I_o
- Absorbance = $\log(I_o/I_t) = -\log(I_t/I_o)$

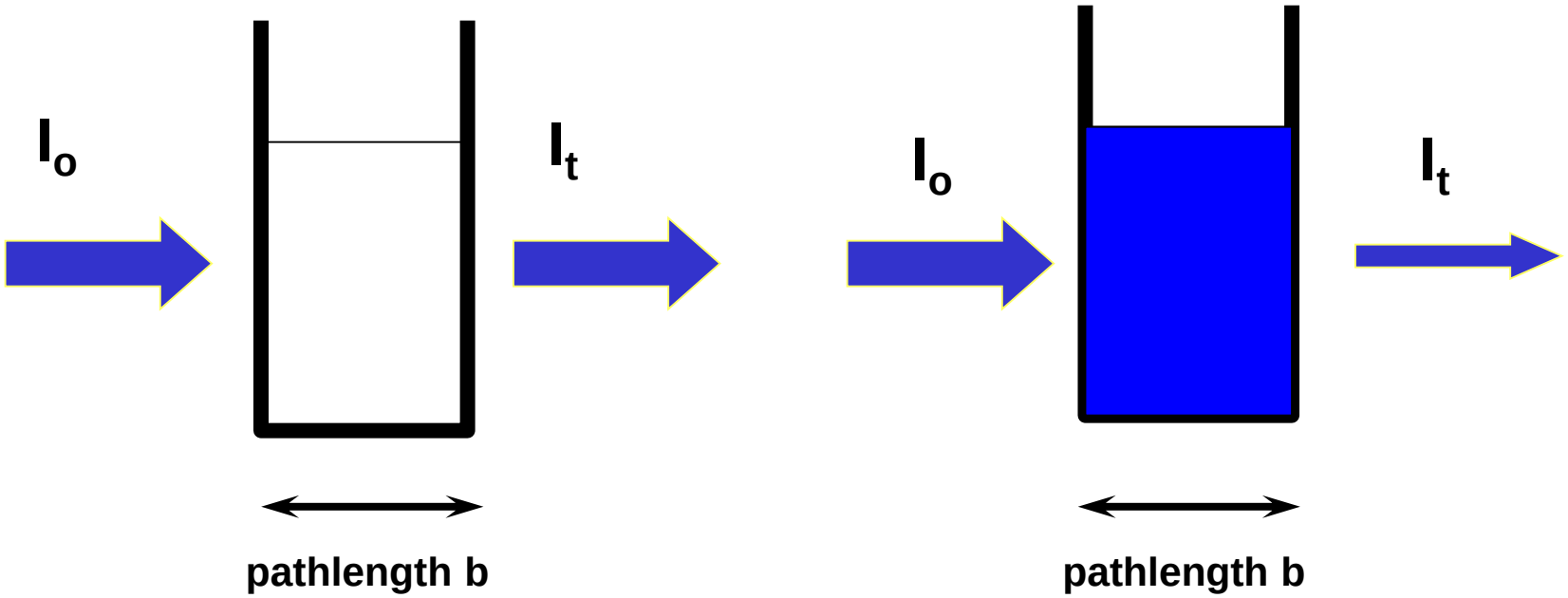


Absorbance & Beer's Law

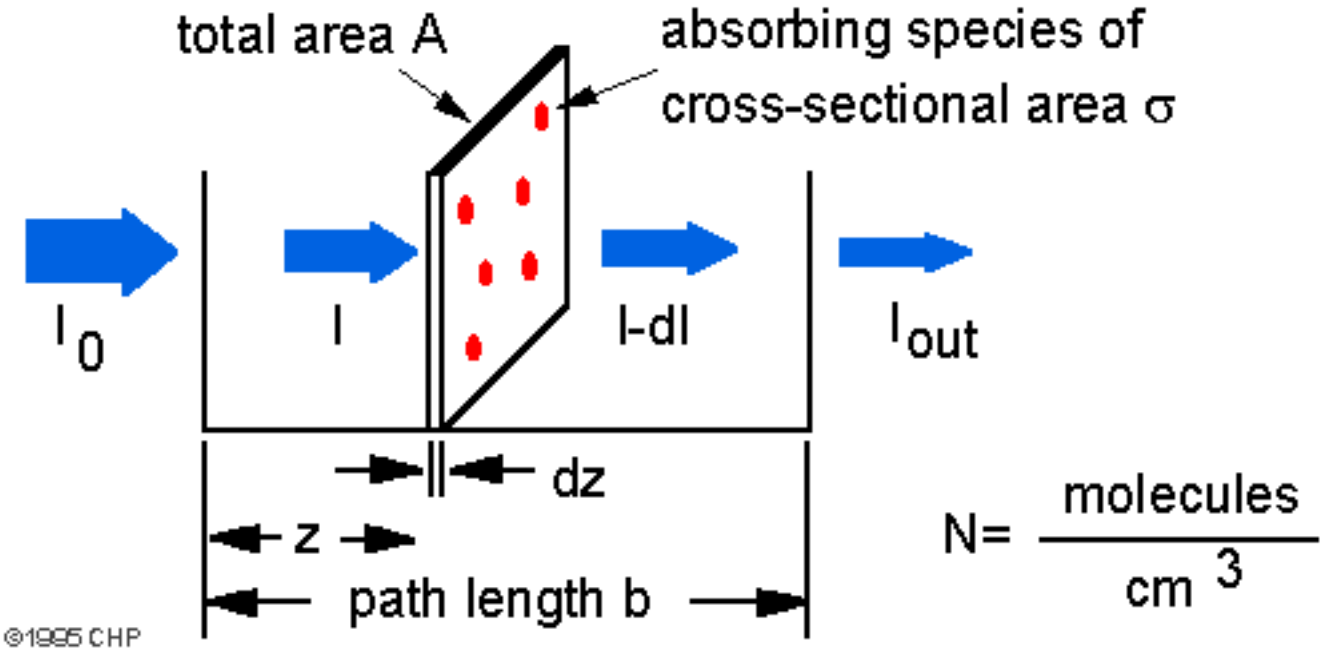


Increasing absorbance

Lambert's Law

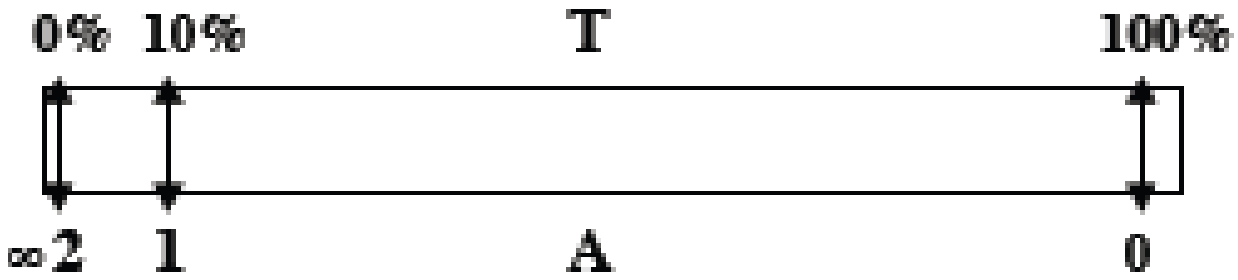


Lambert's Law



Absorbance = εbc

Spectrophotometric Analysis and the Determination of Phosphate Conc.



$$\%T = (\frac{I_t}{I_0}) \times 100\%$$

$$A = -\log(\frac{\%T}{100})$$

$$A = \varepsilon bc$$

Lambert - Beer Law

T-Transmittance

A-Absorbance

ε-molar absorptivity

b-solution path length

c-molar concentration

PROCEDURE-- Part A.1

- ***Organizing your group***

✓ Prepare a group of phosphate solutions with concentrations range from 1.00×10^{-5} M to 4.00×10^{-4} M.

✓ **Each student is responsible for make at least one** of the solutions and measuring at least one data point of Absorbance.

EACH STUDENT MUST PREPARE AT LEAST 1 SAMPLE SOLUTION. ALL E2 IS GROUP EFFORTS EXPERIMENT BUT MUST SUBMIT INDIVIDUAL REPORT. EACH STUDENT MUST COMPLETE 1 ROW OF DATA & ENTER ON THE MAIN DATASHEET. DO ALLPARTS E3: A-E

DO NOT WASTE REAGENTS: **AV** difficult to make, follow rinsing pipet instructions.

Part A: Preparation of 6 Standard Solutions: from 1.00×10^{-5} M to 4.00×10^{-4} M (Pipet *x* mL 0.001M PO_4^{3-} solution, *2.00* mL 2M HNO_3 & *1.00* mL **AV** solution, into 50-mL volumetric flask & dilute with distilled H_2O to mark

Part B: Adjusting the Spectrometer @ λ = 400nm, rinsing the cuvette

Part C: Finding the proper wavelength (λ_{OPT}) for maximum absorbance by using standard stock that has the maximum concentration (stock # 6) and then find its maximum absorbance at varying wavelengths from 400nm to 450nm to find λ_{OPT}

Part D: Making the calibration curve using the standard stock solutions (#1 to # 6) at maximum absorbance (λ_{OPT}) wavelength.

Part E: Determination of the unknown concentration by:

a.a. Using projection from the standard curve.

b.Using slope of the standard curve then Beer's law:

[A = slope x c) & A = - Log(%T/100)]

Make sure to use Excel or Origin software to plot & calculate.

VC211 DATASHEET FOR EXPERIMENT: E3 SPECTROPHOTOMETERY OF PHOSPHOROUS IN THE FORM OF PO_4^{3-}

SECTION: TA: **GROUP EFFORTS &INDIVIDUAL REPORTS: EACH STUDENT SHOULD PREPARE AT LEAST ONE SAMPLE SOL'N, 1 CALIBRATION DATA & COMPLETE ROW DATA**

PART A: PREPARE 6 STANDARD CAL. SAMPLES + 1 UNKNOWN SAMPLE ←----- **PARTS A, D, & E:** SAMPLES PREPARED WITH SHOWN VOLUMES (mL) ------→

PART B: CALIBRATION @ 400nm 0%T(black block)&100%T(Sample 1#) ←**PART A:** CALIBR. SAMPLES WITH SHOWN STNDRD VOLUME SOL'NS→ ←**PART E:** UNKNOWN ABSORB @λ_{OPT}→

←--- **PART C:** FIND λOPT of 6# SAMPLE - ←**PART D:** ABSORBANCE OF PREPARED SAMPLES @λ_{OPT} →

GRP #	NAME	ID #	λ1	λ2	λ3	λ4	λ5	λ_{OPT}	1#	2#	3#	4#	5#	6#	ABSORB. 7#A	ABSORB. 7#B	CONC. (M) 7#A	CONC. (M) 7#B
			400nm	410nm	420nm	430nm	450nm	.....nm	0.00mL	1.00mL	2.00mL	3.00mL	4.00mL	5.00mL	5.00mL	5.00mL	5.00mL	5.00mL
	ABSORBANCE (A)		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	CONC'N	CONC'N
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Maximum Absorbance Optimum Wavelength (λ_{opt})

1. After calibration in Procedure Part B, remove cuvette and rinse in distilled water followed by sample 6# solution. Must use same cuvette, then change mode to absorbance.
2. Insert a 3/4 full of sample 6# solution into the same slot as during calibration with λ = 400nm, then read absorbance (make sure knob is pulled to the location slot of the cuvette).
3. Find the optimum wavelength when maximum absorbance is observed by varying λ=400nm-450nm, always increase knob in same direction and do not go backward until this Procedure Part C is completed. Do not re-calibrate the meter when changing wavelengths.

Important note: the spectrometers in the chemistry building are operating near the low ends of the Gaussians distribution curve of a vs λ, so higher than 400nm will produce erroneous absorbance data that can go up and down but higher that that at 400nm. Therefore, λ_{opt}= 400nm and the remaining absorbance data can be ignored. Many factors can effect this erratic behavior such as: meter light sensitivity near the bottom of the Gaussians curve, accuracy of wavelength dial mechanism, accuracy of dial reading, cuvette condition such as rinsing, cleaning, bubbles, touching, solution reproduction etc.

Additional notes:

a. Make sure to use same cuvette and same slot inside spectrometer compartment

b. Before measuring, rinse cuvette with distilled water 2-3 times and then followed with Sample 6# again 2-3 times.

c. Only hanndle cuvette by the opaque 2 sides and wipe dry carefully withproper tissue before inserting into its slot inside the spectrometer rack.

Procedure Part A: each student prepare 2 samples, group leader 1 sample

Procedure Part B: each student verify calibration

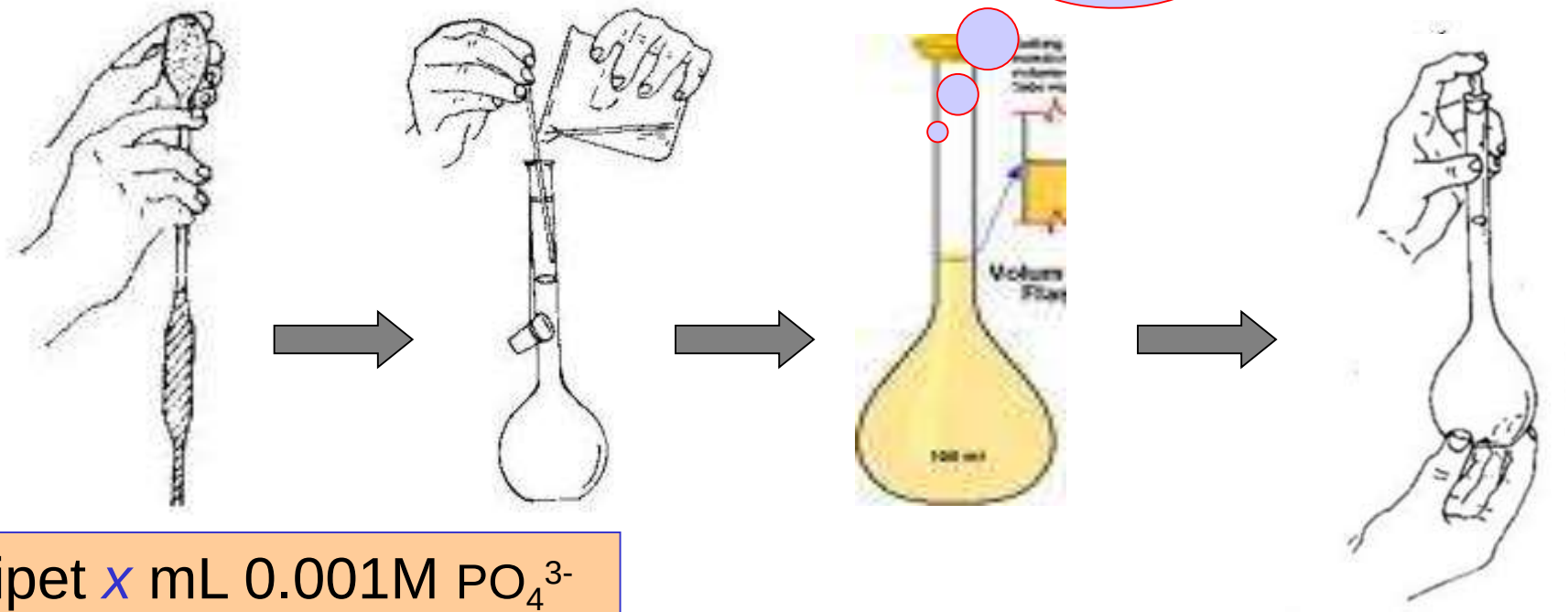
Procedures Part C, D, E: each student measure and record the remaining rows data (A1-A13(or A1-A12 & A14))

1. Wash & rinse all glassware.
2. Rinse each pipet & drive out all excess water then wipe dry the outside. 4 pipets available color coded: 1-mL (yellow tag for AV), 2-mL(black tag for 2M HNO_3), 5-ml (red tag) for 0.001M Na_3PO_4), 5-ml(red tag) for unknown ?M PO_4^{3-}). Keep pipets on the rack each at its original location.
3. Rinse pipet with stock solution (2-3 times). Minimize waste of reagents.
4. Using the pipet 1 mL AV stock solution into each of the 7 volumetric flasks.
5. Repeat step 4 by using 2mL 2M HNO_3 .
6. Repeat step 4 by pipet x mL 0.001M Na_3PO_4 standard solution. See (x) values above. For the unknown molarity sample of PO_4^{3-} dilute 5 mL in a 50.00-mL volumetric using either unknown samples A or B but not both
7. Dilute to 50.00mL mark
8. Measure absorbance of each sample at the optimum λ, make sure you rinse cuvette with each corresponding sample 2-3 time

AV = ammonium vanadomolybdate

Scheme 1

Attention!
Add drop-wise with a pipet!



Pipet *x* mL 0.001M PO_4^{3-} solution, *2.00* mL 2M HNO_3 and *1.00* mL AV solution

Dilute the solution to 50.00mL

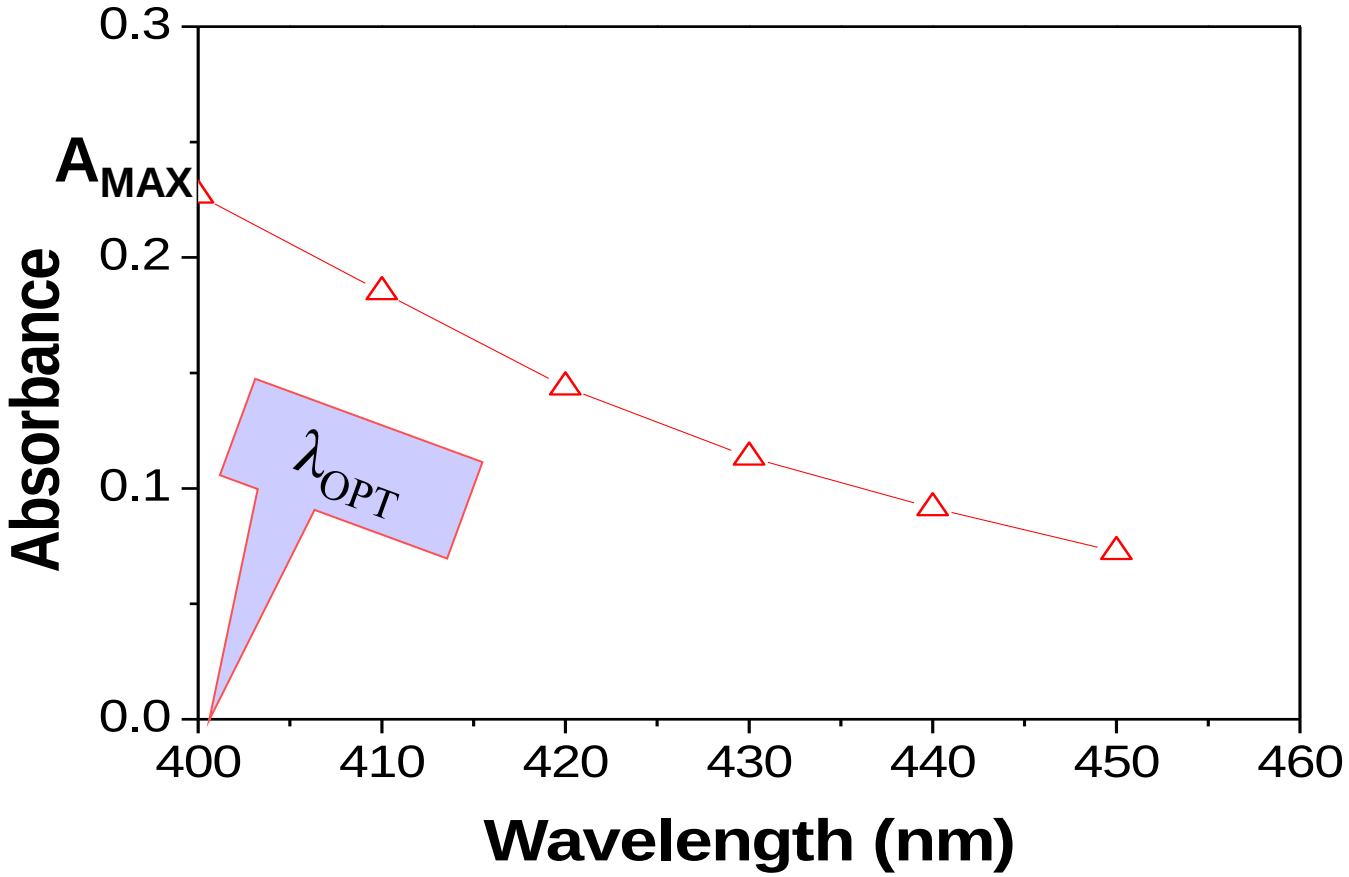
Invert >20 times

PROCEDURE-- Part A.2

- ***Preparation of Standard Solutions***

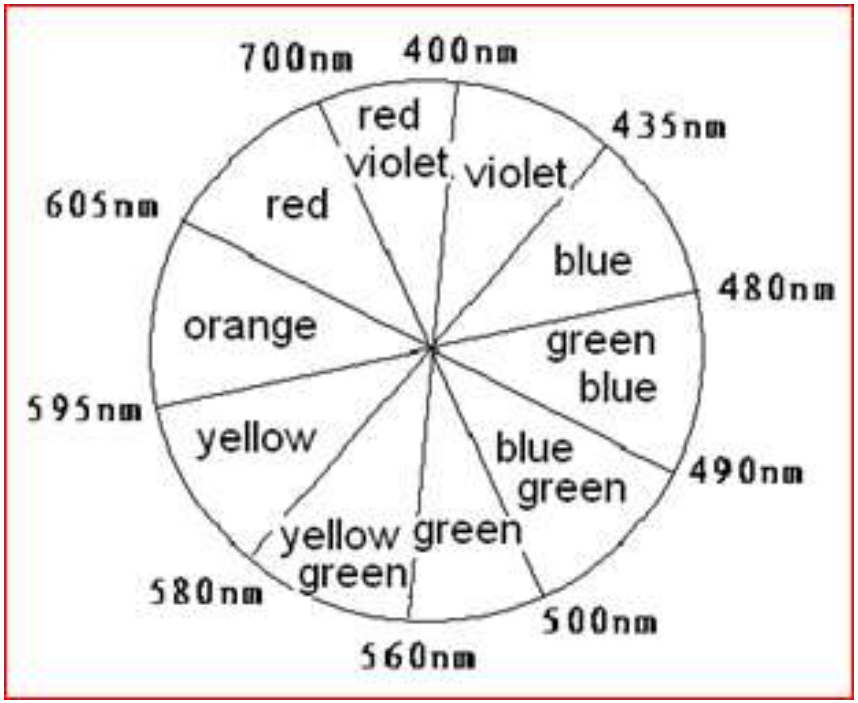
1. Pipet 0.00,1.00,2.00,3.00,4.00,5.00mL 1.00×10^{-3} M phosphate stock solution into $1^{\#}$ - $6^{\#}$ 50-mL volumetric flasks, respectively.
2. Pipet 2.00 mL 2M HNO_3 solution into each $1^{\#}$ - $6^{\#}$ 50-mL volumetric flask.
3. Pipet 1.00 mL of the ammonium vanadomolybdate stock solution into each $1^{\#}$ - $6^{\#}$ 50-mL volumetric flask.
4. Dilute the solution by filling the volumetric flask until the meniscus reach the mark.

The Absorbance Spectrum: **Must generate at least 6 data points & find wavelength λ_{OPT} at maximum absorbance A_{MAX}**

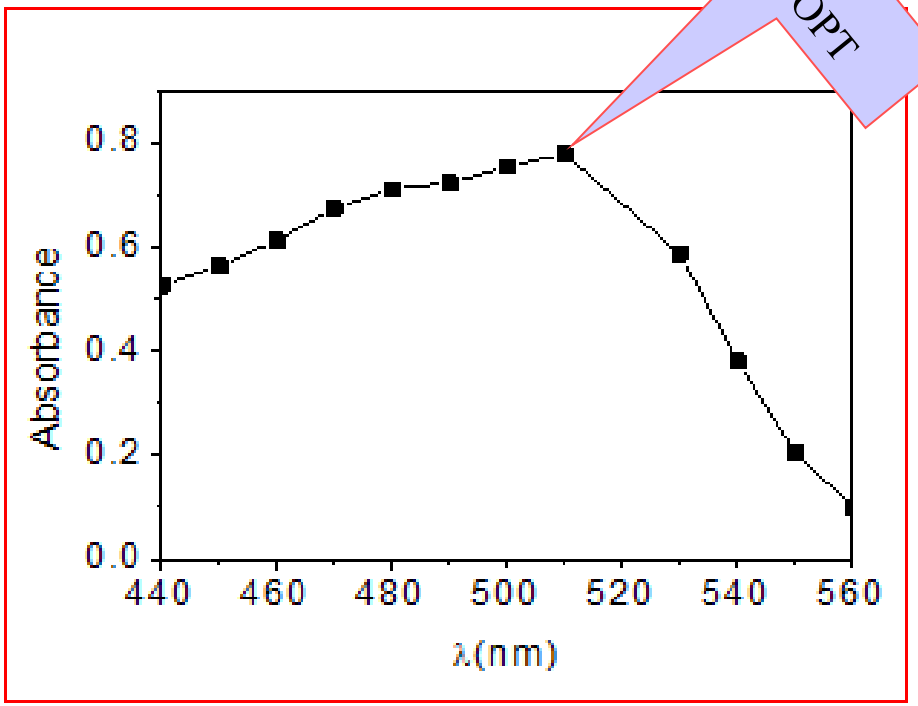


THE ABSORBANCE SPECTRUM

VISIBLE COLOR SPECTRUM WITH LIGHT WAVELENGTH



ION ABSORBANCE CURVE: FIND OPTIMUM WAVELENGTH



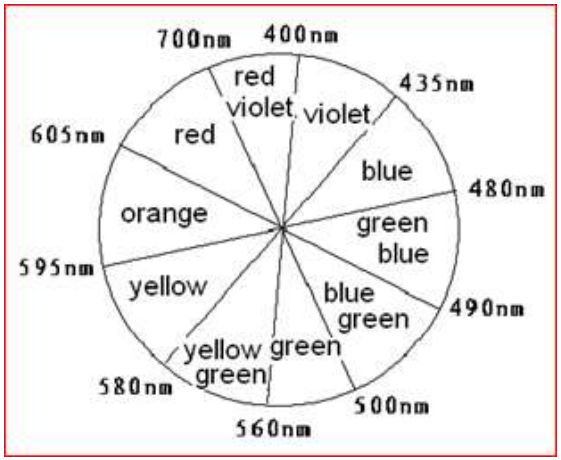
According the complimentary color theory, the color of absorbed light at 520 nm belongs to the GREEN region (which is absorbed), so the color of M^{+n} solution is RED VIOLET (which is reflected into our eyes & see).

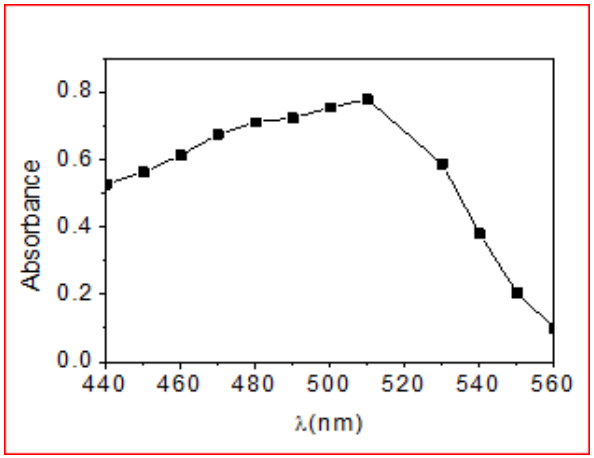
THE ABSORBANCE SPECTRUM

1. Explain % T & A: A = -Log (%T/100%)
2. Graph A & % T, inverted
3. Absorbed vs Observed colors.

VISIBLE COLOR SPECTRUM ION ABSORBANCE CURVE: WITH LIGHT WAVELENGTH FIND MAX. WAVELENGTH

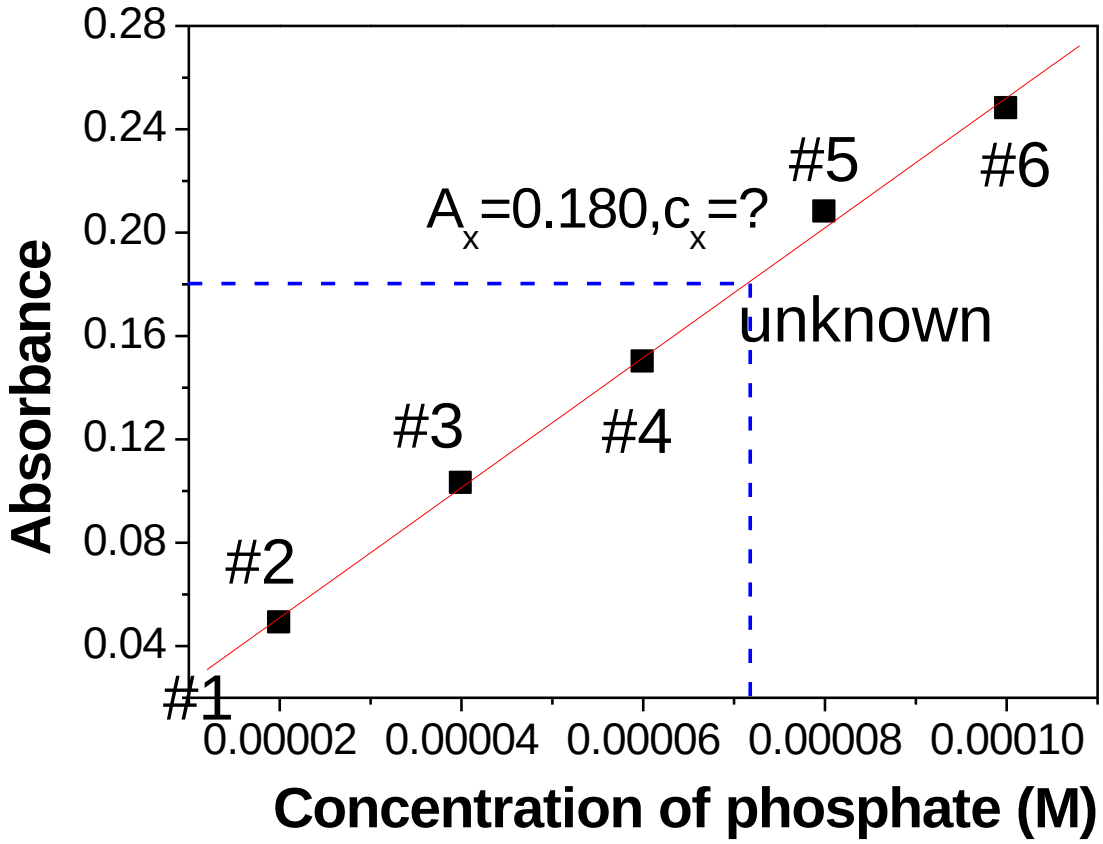
Absorbance vs. wavelength is same as inverted plot for %T vs. wavelength





According the complimentary color theory, the color of absorbed light at 520 nm belongs to the GREEN region (which is absorbed), so the color of M^{+n} solution is RED VIOLET (which is reflected into our eyes & see).

The Calibration Curve



PROCEDURE-- Part B.

- ***Adjusting the Spectrophotometer***

1. Turn on the spectrometer to warm-up (15min).
2. Adjust the wavelenth to 400nm. Use a blackblock to adjust T = 0%.
3. Wash and rinse the cuvettes. Insert a cuvette filled with ¾ blank solution(1#) to set T=100%.

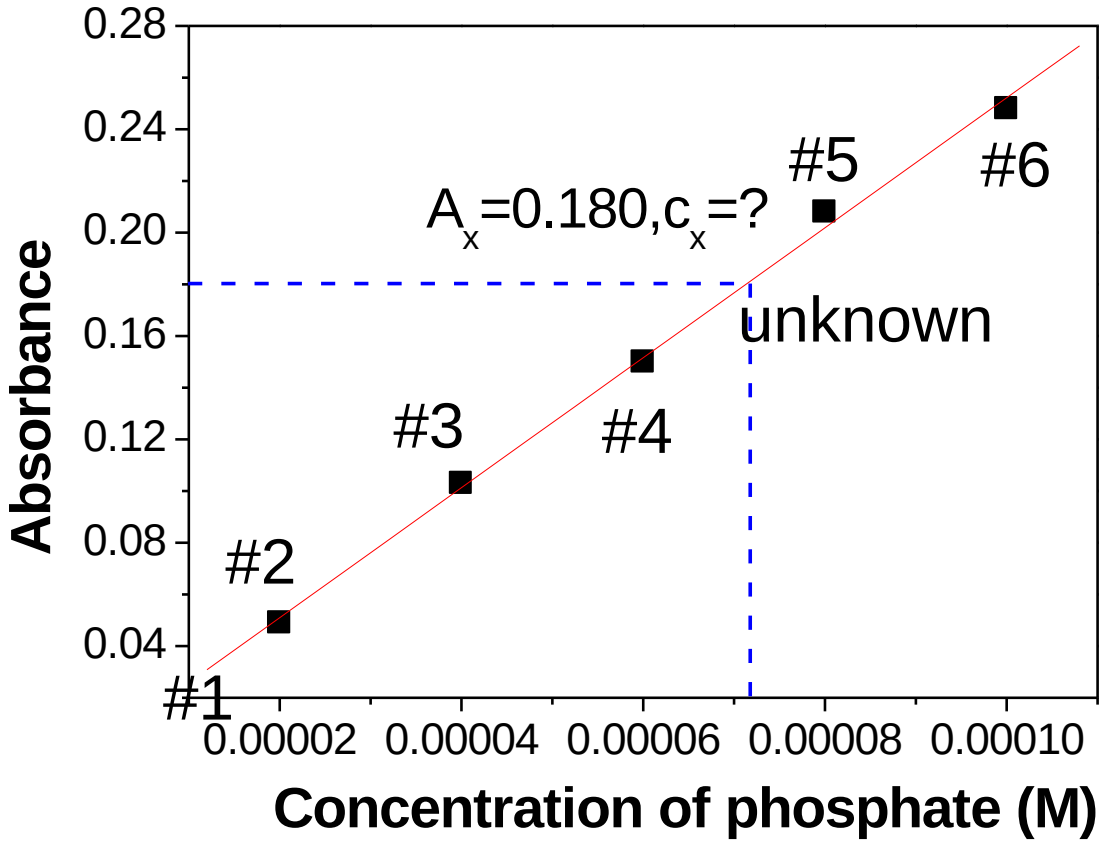
PROCEDURE-- Part D.

- ***Making the Calibration Curve Using the standard Solutions***

1. Rinse the same cuvette, ¾ fill the rinsed cuvette with the 2# solution.
2. Insert the cuvette into the spectrometer. Measure and record A at λ_{max}.
3. Repeat above step for 3# -5# solutions .

- (All data points for a given curve must be measured with the same cuvette)

The Calibration Curve



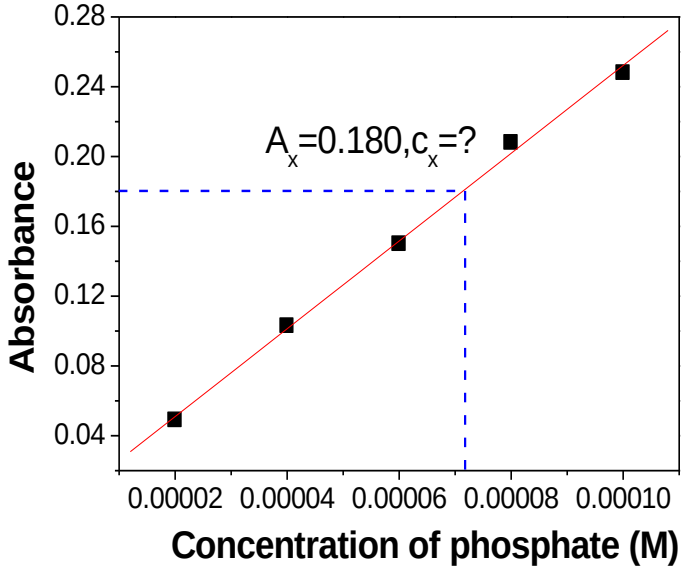
PROCEDURE-- Part E.

- ***Determination of Unknown Concentration***

1. Pipet 5.00 mL of the unknown, 2.00 mL HNO_3 and 1.00 mL of the ammonium vanadomolybdate solution into 7 # 50-mL volumetric flask.
2. Dilute the solution by filling the volumetric flask until the meniscus reach the mark.
3. ¾ fill the rinsed cuvette with the unknown solution. Use the spectrometer to measure A.
4. Determination of unknown concentration by using the calibration curve.

Data?

- Make the curves using a computer
- Use software, such as excel or origin



How to Use a Spectrophotometer

- **Spectrophotometers are used to determine absorbency at certain wavelength of a solution. This can then be used to determine the concentration of a solution or determine what an unknown substance is.**

- **A digital spectrometer measures the amount of visible light absorbed by a colored solution. This can be read as Absorbance or % Transmittance.**

Setting Up the Spectrometer

- **First, check that the instrument is turned on. You will hear the fan and see light coming out of the right side of the instrument.**
- **Next press the "MODE" button to select % Transmittance mode. The current mode appears on the display.**

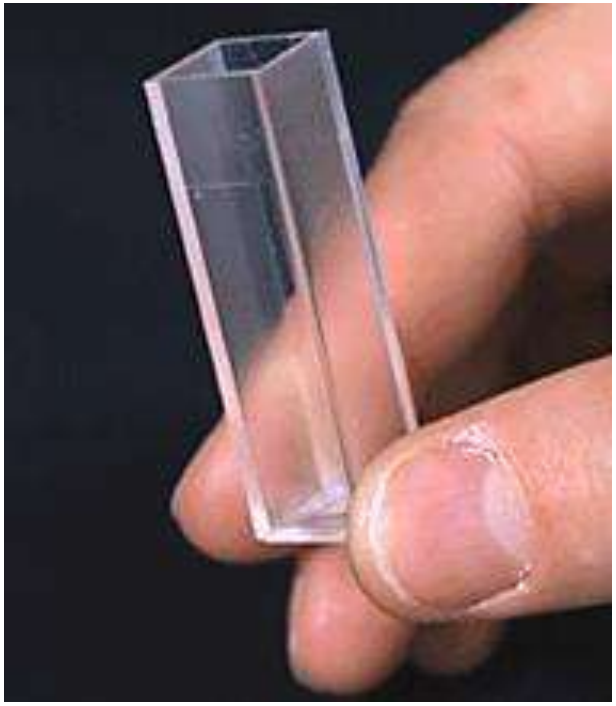
How to Use a Spectrophotometer

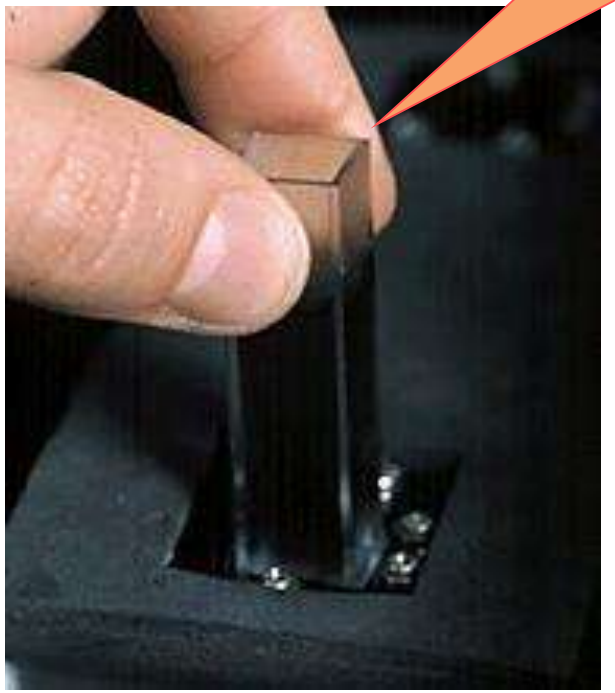
- **Set the wavelength to the desired value using the knob on the top.**
- **Open the cover, press 0% T to set the absorbance of the background to 0%T.**
- **Fill a cuvette (3/4 full) with your blank solvent and dry the outside of the cuvette carefully (make sure cuvette is first pre-rinsed 3 times with few mL of blank solvent.**
- **Insert your blank cuvette in the first compartment and close the cover.**
- **Press 100% T to set the Transmittance of the blank to 100%T.**

How to Use a Spectrophotometer

- **Insert a dry sample cuvet in the second compartment and close the cover, pull the draw bar at the first step. Press the "MODE" button to select Absorbance mode. The absorbance value of the sample will now be shown on the LCD.**

cuvette





Rinse 3 times and ¾ fill with the solution

Dry the outside of the cuvette with a tissue and insert it into the spectrometer