Spectrophotometric Analysis

Professor Thomas Hamade,
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&

Department of Chemistry, SJTU

(shortest experiment: 2:15)


The most handsome one is Harry!
The big face boy is Rickson!
The small-eye guy is Mickey!


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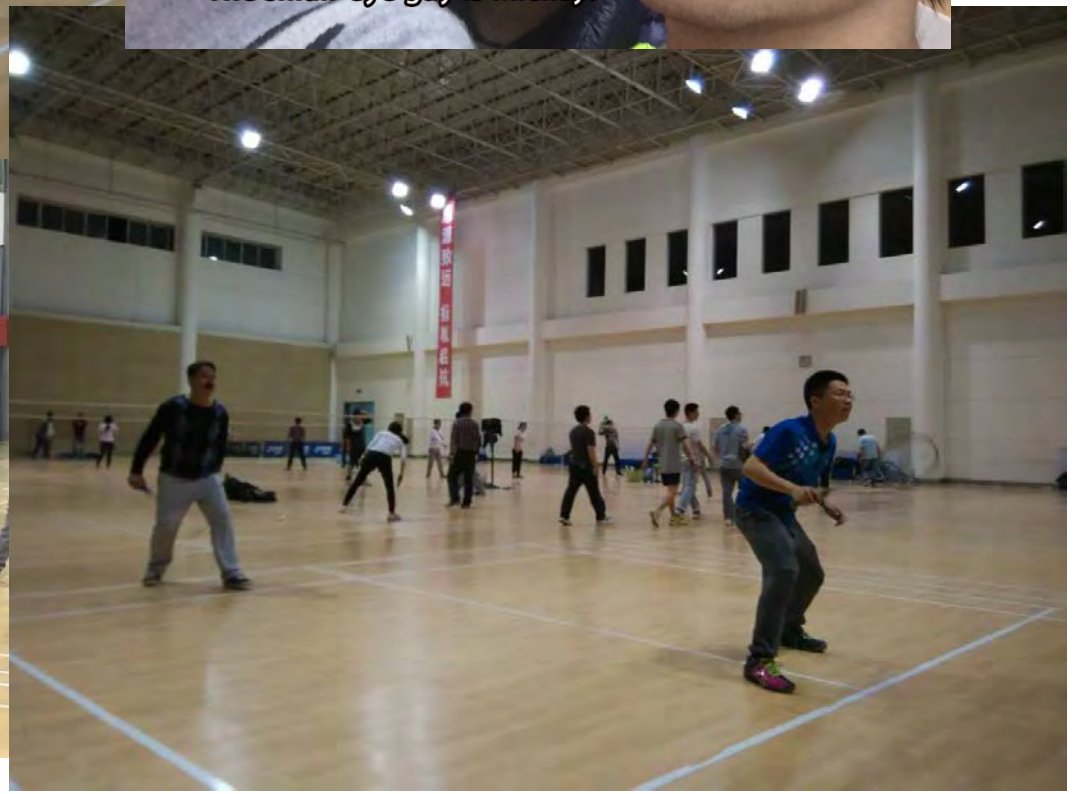

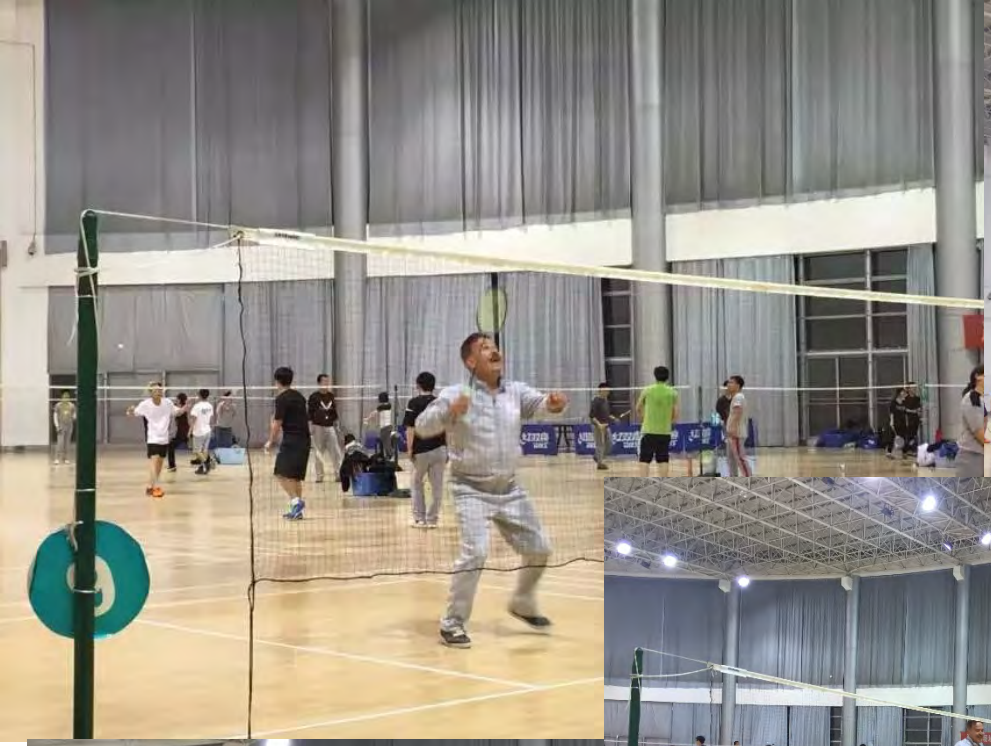



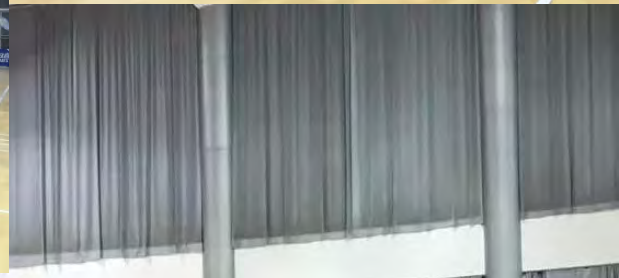

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Huashan WORLDWIDE Medical Center

SICK LEAVE CERTIFICATE
病 假 证 明

Patient's Name 姓名: Homade Thomas Gender 性别: M

Date of Visit 就诊日期: March 23, 2015 DOB生日: June 4, 1954

Out-patient Number 门诊号: 2014 - 01999

Diagnosis 诊断: Lumbar disc protrusion is suspected

Recommendation 建议:

Seven Days/weeks天/周 Sick leave 休息:

From从 March 23, 2015 To到 March 29, 2015

HUASHAN HOSPITAL MEDICAL CENTER

Doctor Signature
医师签字

March 23, 2015
Date Written
日 期

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SHANGHAI 200040 P.R.China
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E-mail: Clinic@sh-hwmc.com.cn
In-patient@sh-hwmc.com.cn
上海华顺医院有限公司

SAFETY HIGHLIGHTS

☐YOU ARE RESPONSIBLE FOR YOUR OWN SAFETY FIRST THEN OTHERS

☐BROKEN GLASS, PREVENTION& DISPOSAL ☐HOW TO WASH & RINSE GLASSWARE ☐WEAR GOGGLES & LAB COATS

☐KEEPGLASSWARE AT LEAST 20cm AWAY FROM EDGE OF BENCH

☐CLUTTER (MESS)

☐CHEMICAL WASTE & DISPOSAL (ORGANIC, INORGANIC &, CORROSIVES, & SOLIDS) ☐WASTING CHEMICALS BE CONSERVATIVE & PROTECT ENVIRONMENT☐IMMEDIATELY STORE AWAY STOCK CHEMICALS (COVER ON TIGHTENED & TOP BENCH)

☐SAFETY RUBBER GOLVES (CORROSIVE LIQUIDS RESISTANT & SOLVENTS RESISTANCE)

☐SAFETY CLOTH GLOVES & TONGUES: HEAT PROTECTION, HOT PLATES, & BURN PROTECTION ☐FIR HAZARDS & PROTECTION (EXTINGUISHERS) ☐SPATULAS ☐CHEMICAL TRANSPORTATION PROHIBITED, NOT EVEN ALLOWED TO TAKE OUTSIDE THE DOOR

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)]

- AVOID WASTING CHEMICALS
- DO NOT REPEAT MAKING ANY SAMPLE SOLUTIONS, NOT EVEN IF YOU MAKE A MISTAKE – DISCUSS YOUR SITUATION IN THE REPORT
- USE ONLY ONE UNKNOWN (A) OR (B) AS DIRECTED BY INSTRUCTOR BUT NOT BOTH
- DO NOT DISPOSE ANY CHEMICAL WASTE INSIDE THE SINK, INSTEAD YOU MUST USE THE PROPER DISPOSAL CONTAINER

BACKGROUND

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

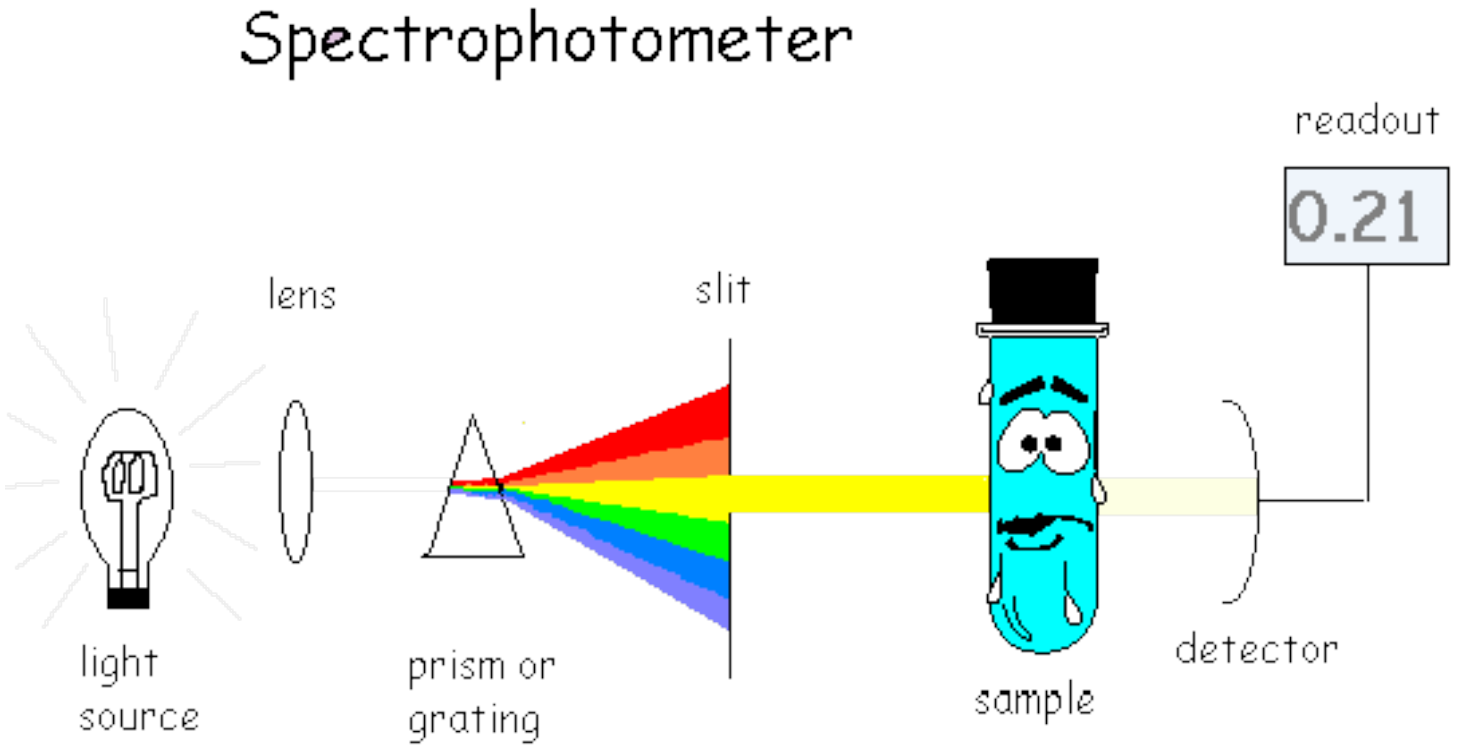
Chromogenic Reaction of Phosphate

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

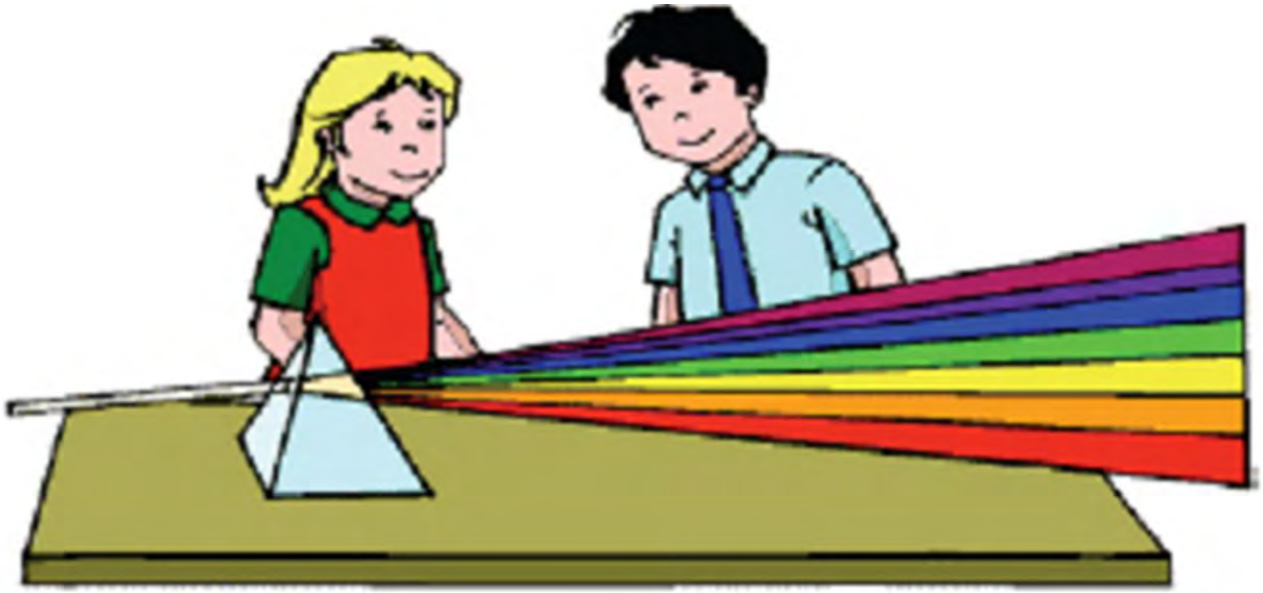
$$\rightarrow \quad (NH_4)_3PO_4 \cdot NH_4VO_3 \cdot 16MoO_3$$

Chromogenic Reagent, **AV** solution

(1) Spectrophotometric Analysis and the Determination of Phosphate



(2) Spectrophotometric Analysis and the Determination of Phosphate



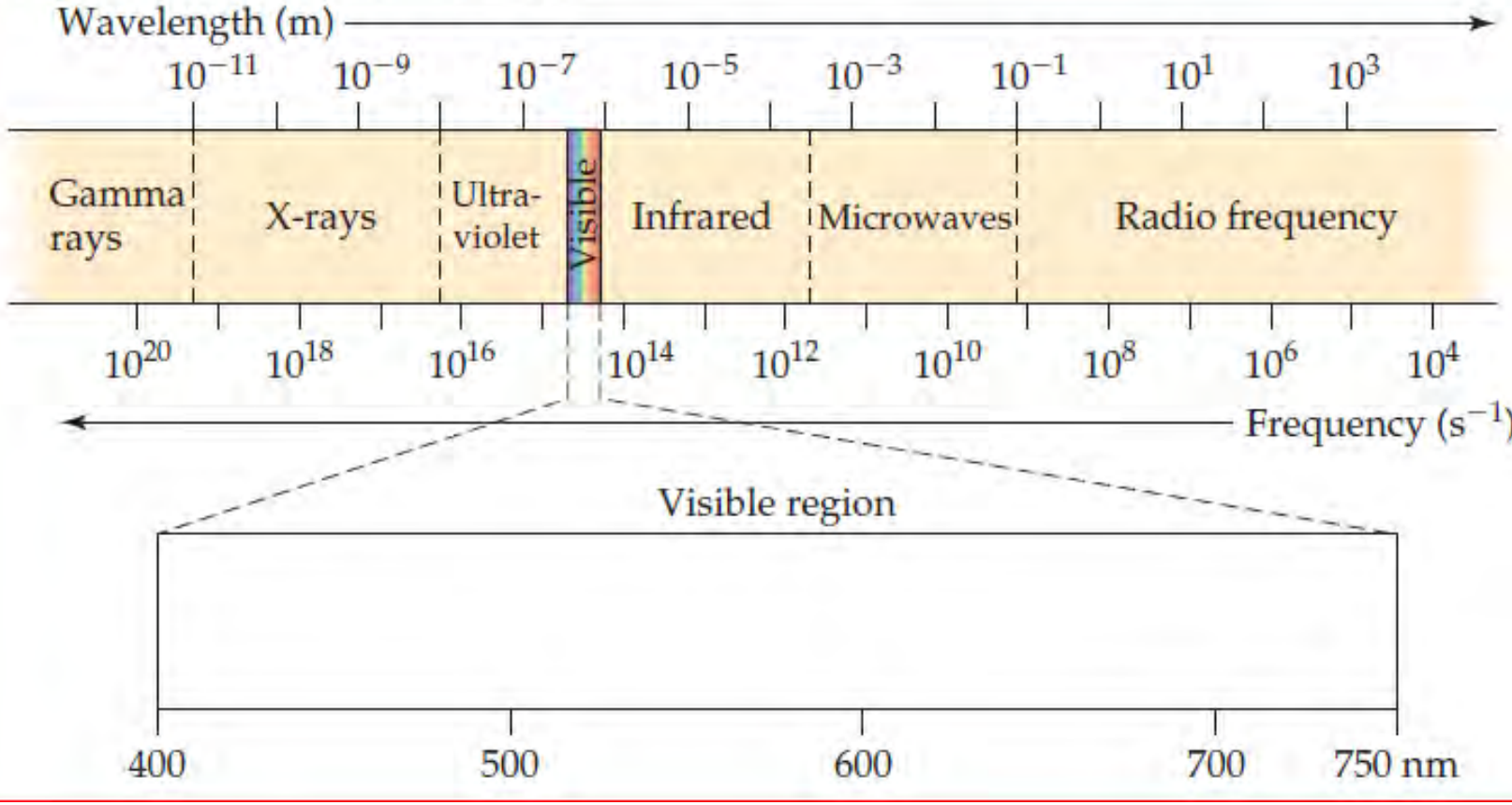
λ 400 Violet - Blue - Green - Yellow - Orange - Red λ 800

←

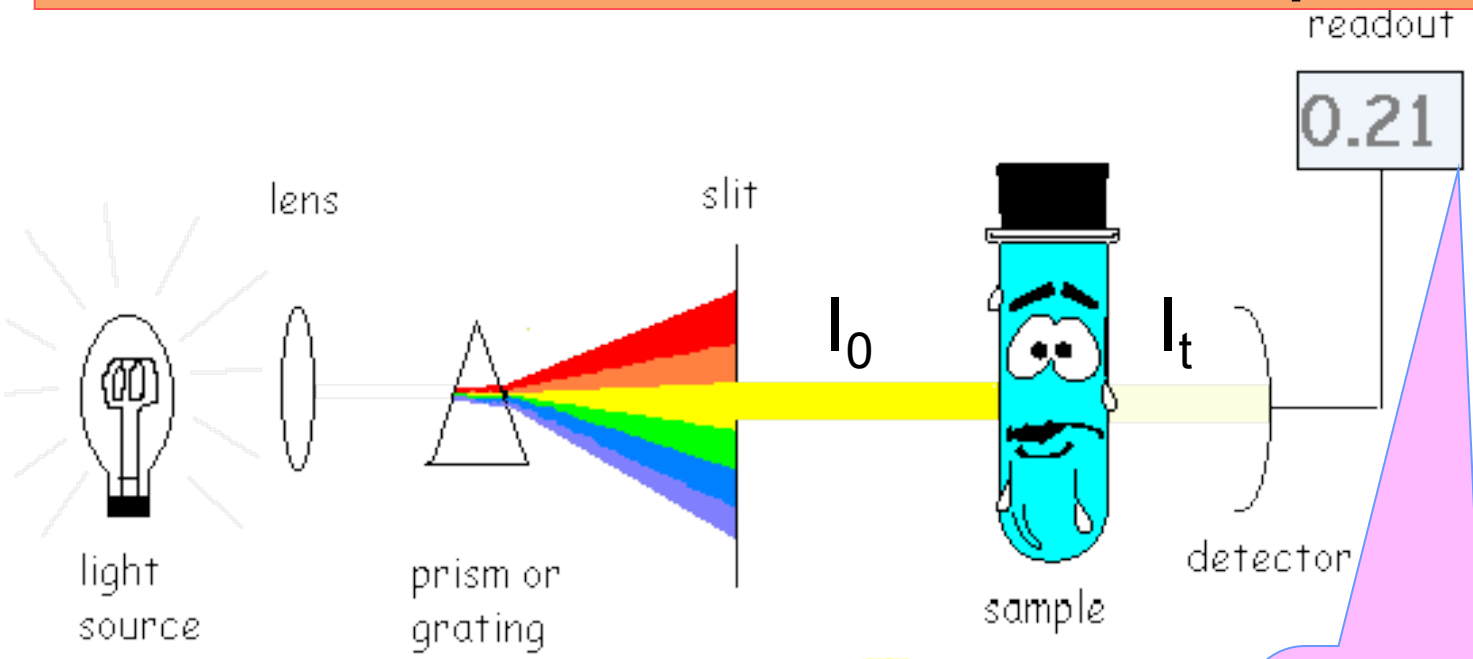
Shorter wavelength
Higher frequency
Higher quantum energy

LIGHT SPECTRUM

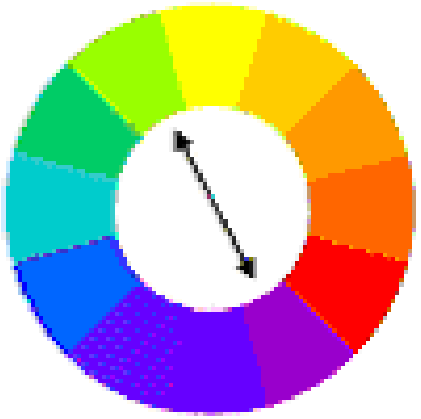
How do the wavelength and frequency of an X-ray compare with those of the red light from a neon sign?



(3) Spectrophotometric Analysis and the Determination of Phosphate



Complimentary color

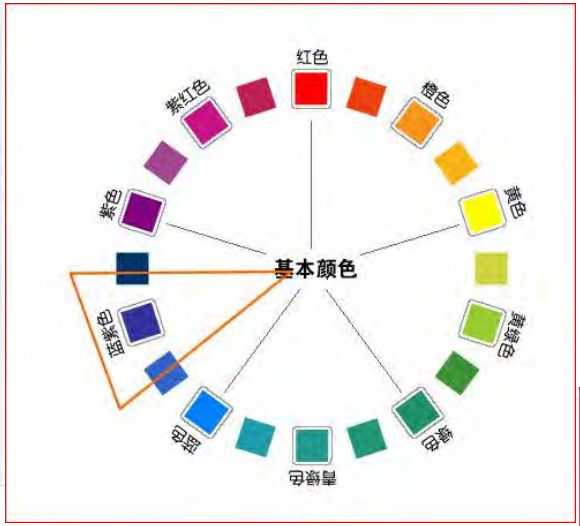


Color absorbed or transmitted?

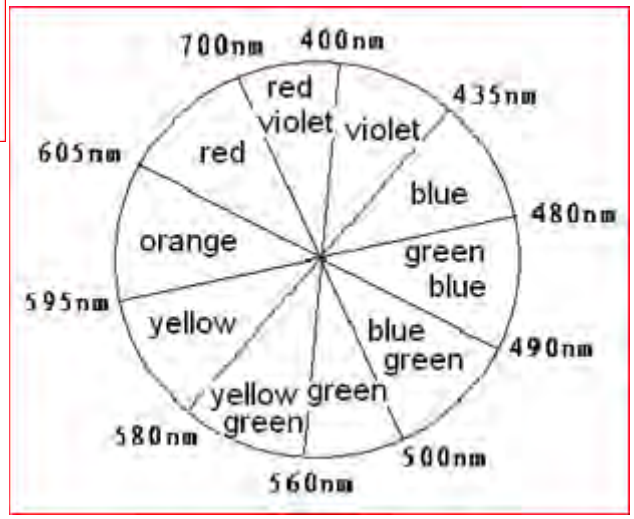
PROCEDURE-- Part B.

COMPLIMENTARY COLORS

VISIBLE LIGHT SPECTRUM

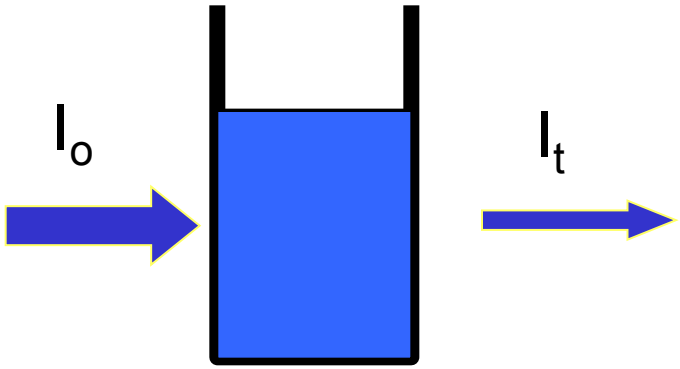


VISIBLE LIGHT WAVELENGTH

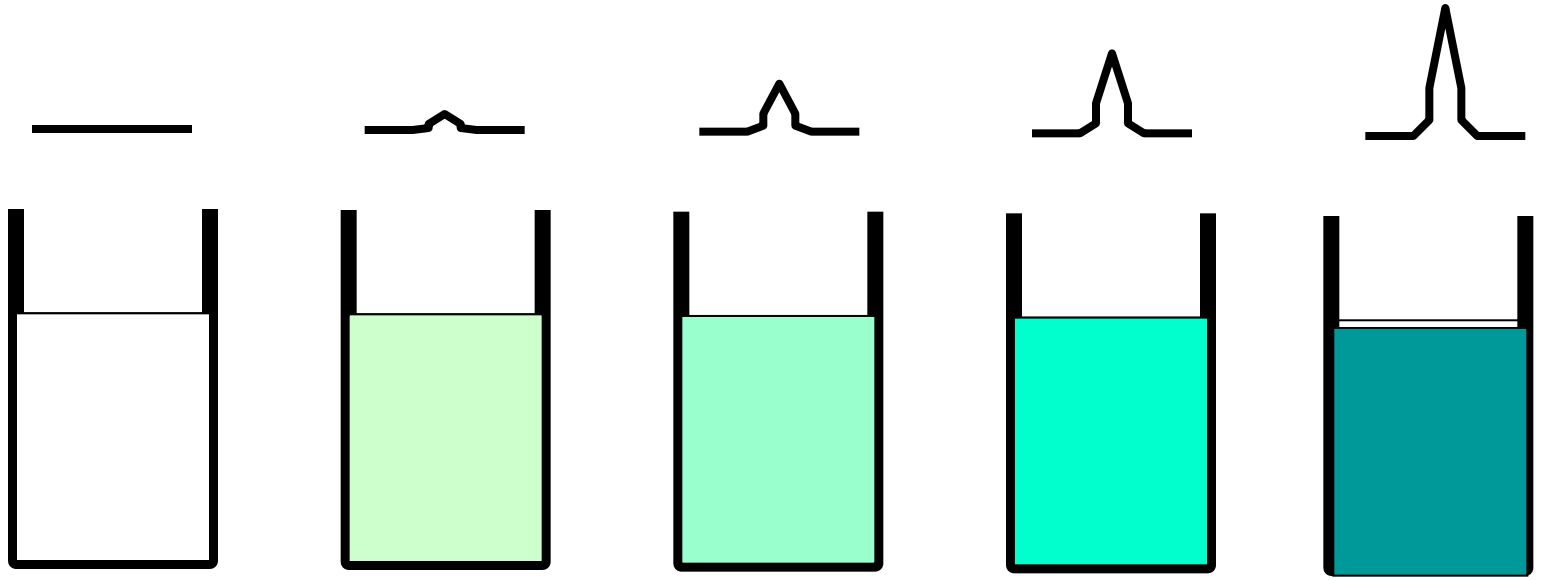


Lambert—Beer Law

- 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 = $\log(I_o/I_t) = -\log(I_t/I_o)$

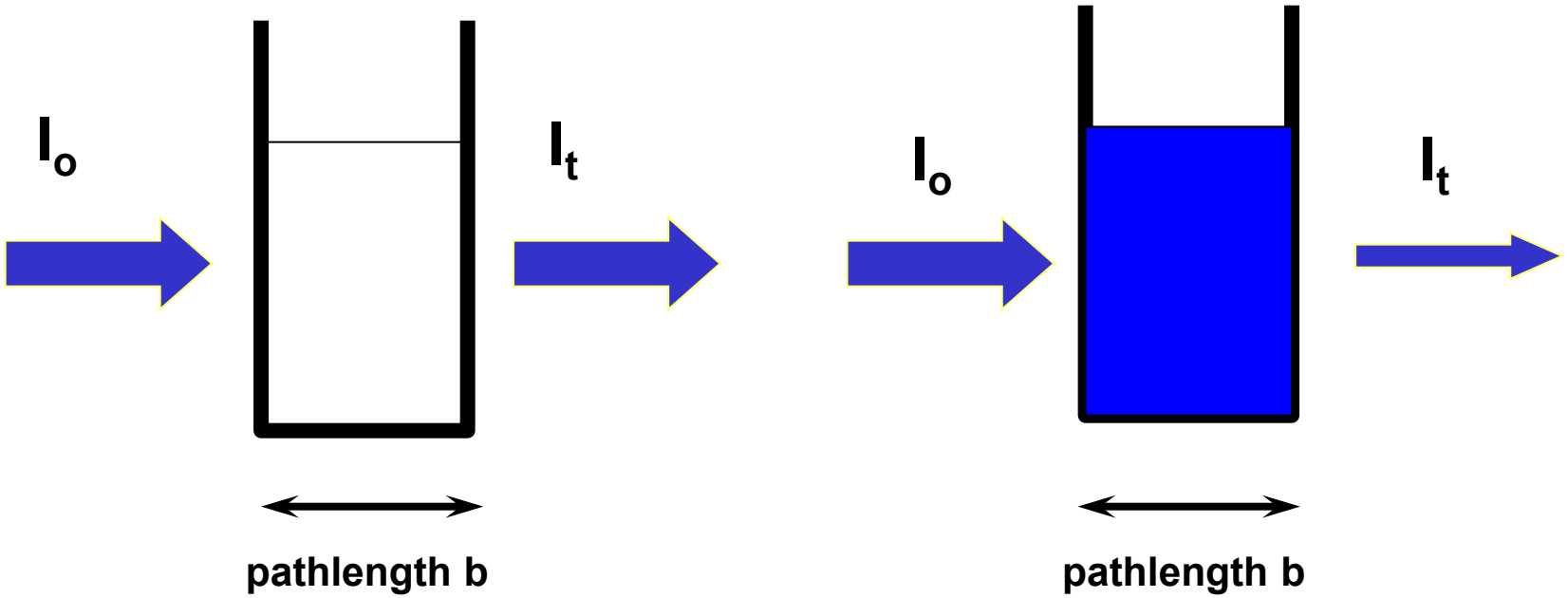


Absorbance & Beer's Law

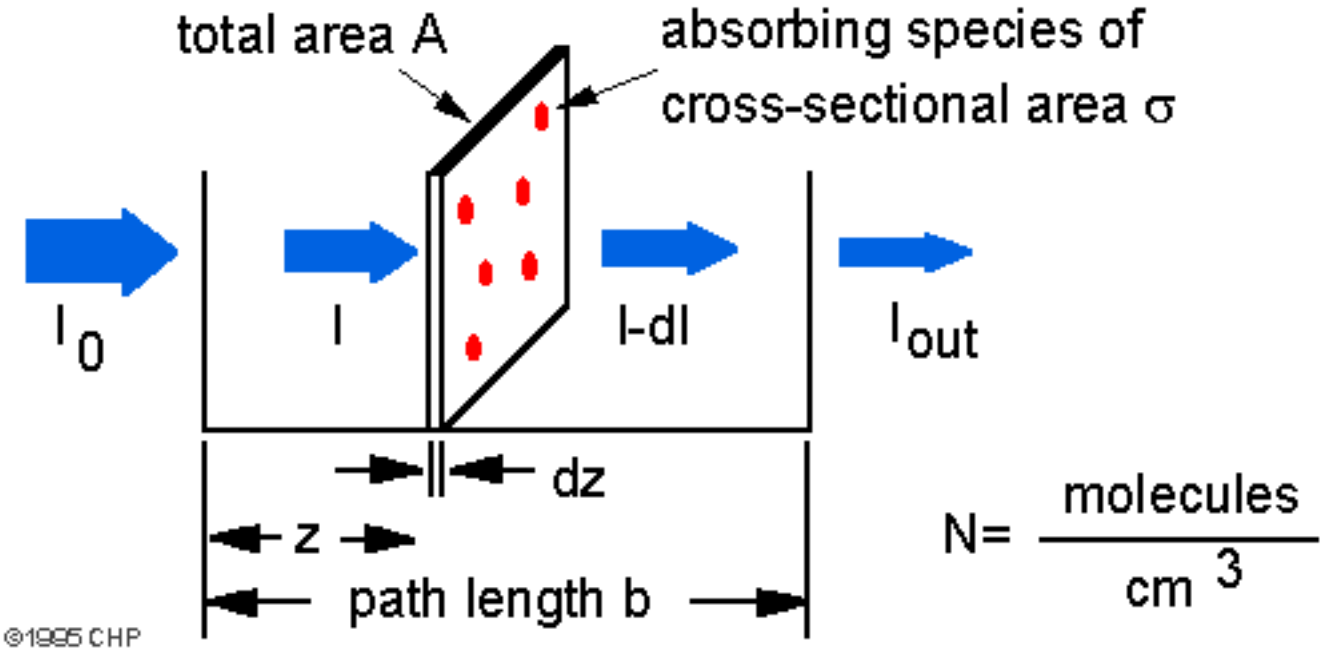


Increasing absorbance

Lambert's Law

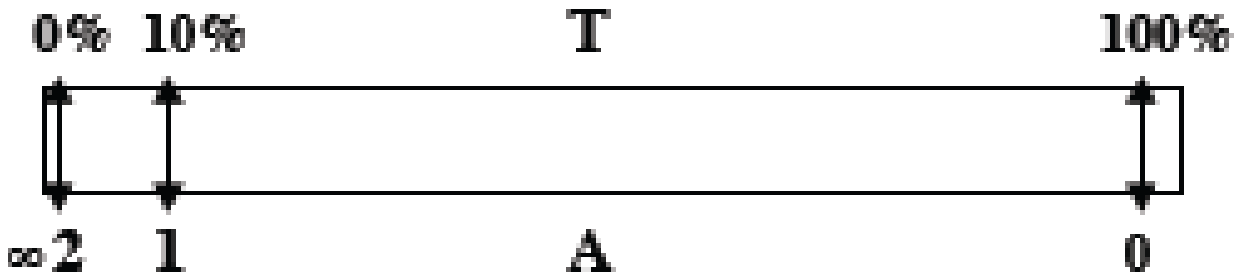


Lambert's Law



Absorbance = εbc

(4) 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

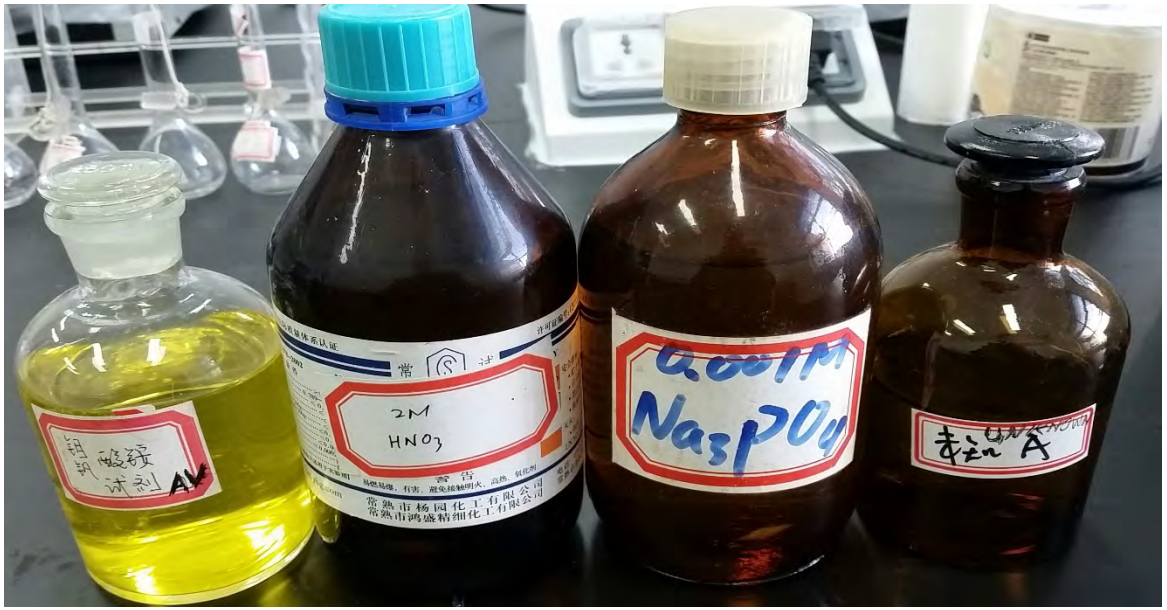
PROCEDURE-- Part A.1

- ***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.

HANDLE CHEMICALS & GLASSWARE CAREFULLY & WASH HANDS AFTER HANDLING



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 ALL PARTS 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 (i.e. Sample 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 (Sample 1# to 6#) at maximum absorbance (λ_{OPT}) wavelength.

Part E: Determination of the unknown concentration by:

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)]

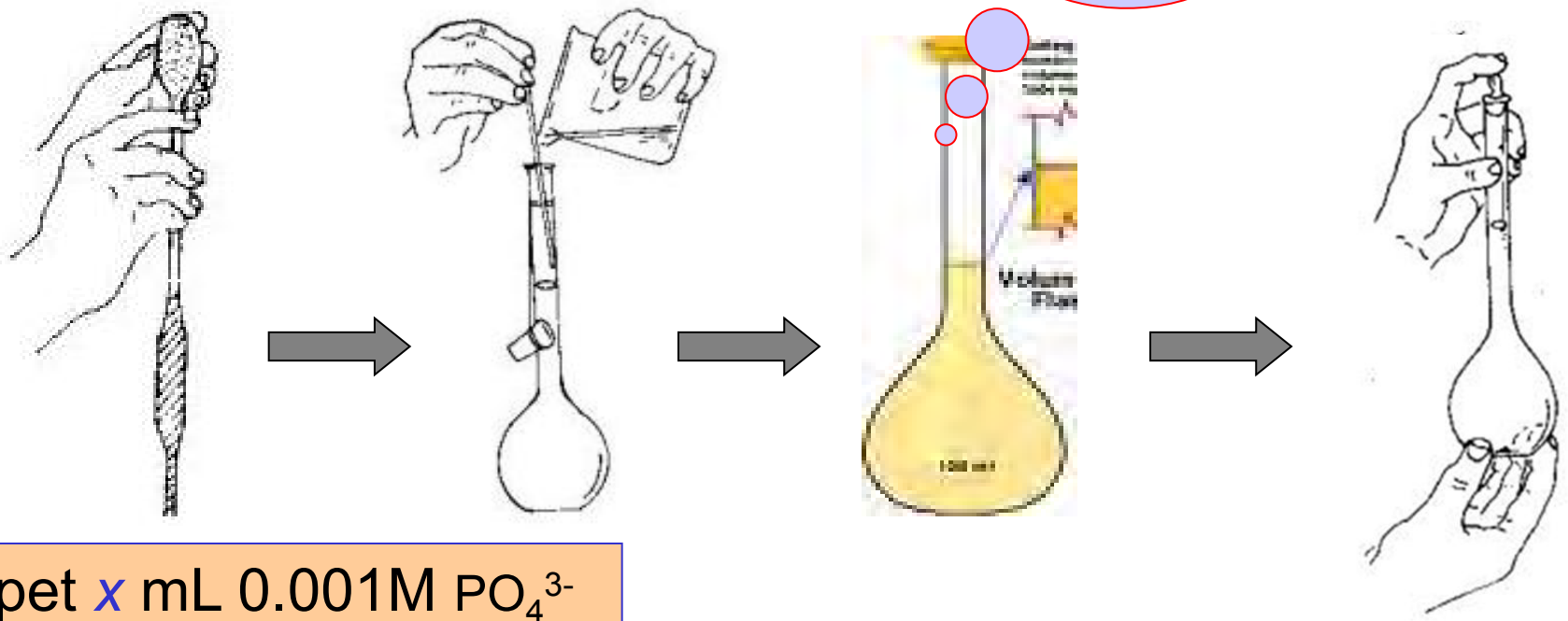
Make sure to use Excel or Origin software to plot & calculate.

TIPS TO RINSING GRADUATED PIPETTE

- ***Wash under sink, wash with few mL distilled water using wash bottle while swirling around. Push excess water from inside using hand bulb pump. Wipe clean the outside and the tip.***
- ***Carefully immerse to about 4cm into the stock solution so solution seeps inside the pipette. Then draw pipette out and swirl the inside solution to rinse the inside moisture. Push excess solution out using the hand bulb into chemical waste beaker.***
- ***Immerse pipette into the stock solution and draw solution about 1/3 way, then swirl around the inside liquid over the waste beaker to make sure pipette is properly rinsed. Now the pipette is ready to insert into stock solution. Use same pipette for the remains of the samples.***
- ***Dispose the waste chemicals into the proper waste container.***

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.

GROUP EXPERIMENT

PROCEDURE-- Part A.

- *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. (Addition Volume and concentration of phosphate in prepared solutions)

sample	$1^{\#}$	$2^{\#}$	$3^{\#}$	$4^{\#}$	$5^{\#}$	$6^{\#}$
V (mL)	0.00	1.00	2.00	3.00	4.00	5.00
c (M)						

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.

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.**
- **Follow the procedures in the following slides**

Handling the cuvette:

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.

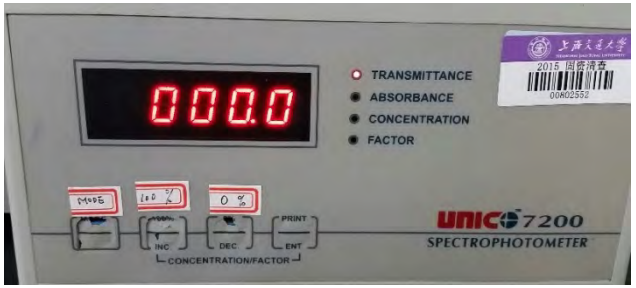
c. Only handle cuvette with the opaque side and wipe dry before inserting into spectrometer.

cuvette

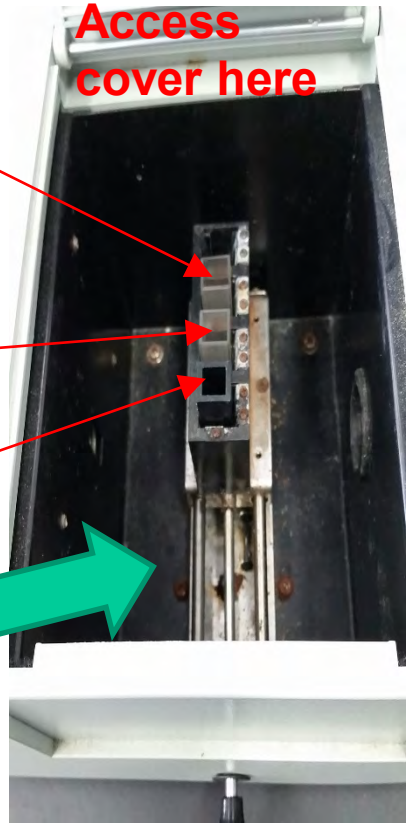
Rinse 3 times and ¾ fill with the solution

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

TYPICAL SPECTROMETER SETUP



6. Use Sample 6# cuvette (5mL PO_4^{-3}). Change mode to Absorbance. Record [A1]
5.Use a blank Sample 1# cuvette (0mL PO_4^{-3}) to adjust T = 100.0% if different than display.
4.Use a black block to adjust T = 000.0% if different than display.
3.Insert black block and the 2 cuvettes (3/4 full) into the 3 slots shown
2. Adjust the λ to 400nm & mode to Transmittance
1.Turn power on (15 min.)



7.Find λ_{OPT}: Adjust λ to 410nm, repeat steps 4-6. Record [A2]
8.Repeat step 7 at λ = 420-450nm. Record corresponding A3-A6
9. Find λ_{OPT} then adjust dial λ to λ_{OPT}. Do not touch the dial any more. Repeat steps 4-6 at λ_{OPT}. Record [A12].
10. Replace Sample 6# cuvette with Sample 2# cuvette (1mL 5mL PO_4^{-3}). Repeat steps 4-6 but using the replacement cuvette instead of cuvette in Step 6. Read [A8].
11. Repeat Step 10 for the remaining samples [A9-A12 or A9-B12].

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

PART B: CALIBRATION @ 400nm 0%T(black block)&100%T(Sample 1#)

←----- **PARTS A, D, & E:** SAMPLES PREPARED WITH SHOWN VOLUMES (mL) ----->

←**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	BLANK A7	A8	A9	A10	A11	A12	A13	A14	CONC'N	CONC'N
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TYPICAL SPECTROMETER SETUP

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4. Use a black block to adjust T = 000.0% if different than display.
3. Insert black block and the 2 cuvettes (3/4 full) into the 3 slots shown
2. Adjust the λ to 400nm & mode to Transmittance
1. Turn power on (15 min.)

7. Find λ_{OPT}: Adjust λ to 410nm, repeat steps 4-6. Record [A2]
8. Repeat step 7 at λ = 420-450nm. Record corresponding A3-A6
9. Find λ_{OPT} then adjust dial λ to λ_{OPT}. Do not touch the dial any more. Repeat steps 4-6 at λ_{OPT}. Record [A12].
10. Replace Sample 6# cuvette with Sample 2# cuvette (1mL 5mL PO_4^{-3}). Repeat steps 4-6 but using the replacement cuvette instead of cuvette in Step 6. Read [A8].
11. Repeat Step 10 for the remaining samples [A9-A12 or A9-B12].

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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. 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.

PROCEDURE-- Part B

Refer to previous slide diagrams

- ***Adjusting the Spectrophotometer***

1. Turn power on (15 min.)

2. Adjust the dial knob wavelength λ to 400nm & mode to Transmittance

3. Insert black block cuvette & 2 cuvettes (3/4 full) into the first bottom three 3 slots of the cuvette holder

4. Pull/push sliding knob to the black block position (1st slot of the cuvette holder), then adjust T = 000.0% if different than display.

5. Position the slide knob to the blank Sample 1# cuvette (0mL PO_4^{-3}) (2nd slot of the cuvette holder), then adjust T = 100.0% if different than display.

PROCEDURE-- Part C
Refer to previous slide diagrams

- ***Making the Absorbance Spectrum***

6. Position slide knob to Sample 6# cuvette (5mL PO_4^{-3}) (3rd slot of the cuvette holder). Change mode to Absorbance. Record [A1]

7. Find λ_{OPT}: Adjust λ to 410nm, repeat steps 4-6. Record [A2]

8. Repeat step 7 at λ = 420-450nm. Record corresponding A3-A6 (see next slide).

9. Find λ_{OPT} then adjust dial λ to λ_{OPT}. Do not touch the dial any more. Repeat steps 4-6 at λ_{OPT}. Record [A12].

PROCEDURE C: **GENERATE AT LEAST 6 DATA POINTS**

- Preparation of solutions (7)
- Measuring **A**bsorbance of every solution

λ(nm)	400	410	420	430	440	450
A						

$\lambda_{Opt} = ?$

PROCEDURE-- Part D & PART E

- ***Making the Calibration Curve Using the standard Solutions & Measuring the Unknown Absorbance***

10. Replace Sample 6# cuvette with Sample 2# cuvette (1mL 5mL PO_4^{-3}). Repeat steps 4-6 but using the replacement cuvette instead of cuvette in Step 6. Read [A8].

11. Repeat Step 10 for the remaining samples [A9-A12 or A9-B12].

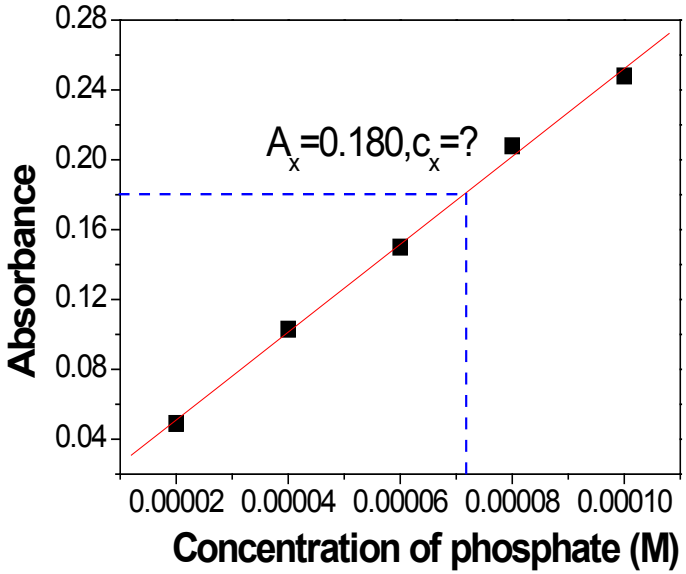
PROCEDURE: Part D & Part E

- **Data** $\lambda = \lambda_{max}$

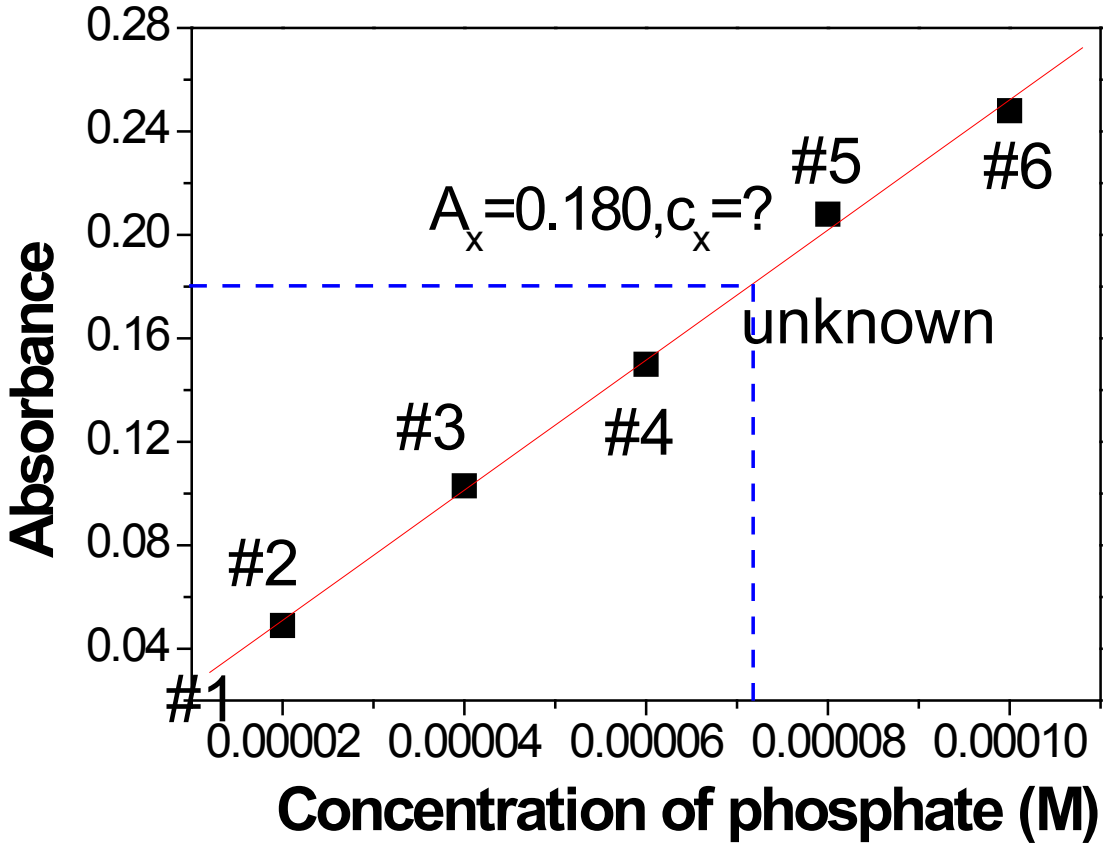
	2#	3 #	4 #	5 #	6 #	7 # Unknown
C(M)						
A						

Data?

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



The Calibration Curve



Notes for PROCEDURE-- Part E.

- ***Preparation & Determination of Unknown (A or B) Concentration***

a. 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.

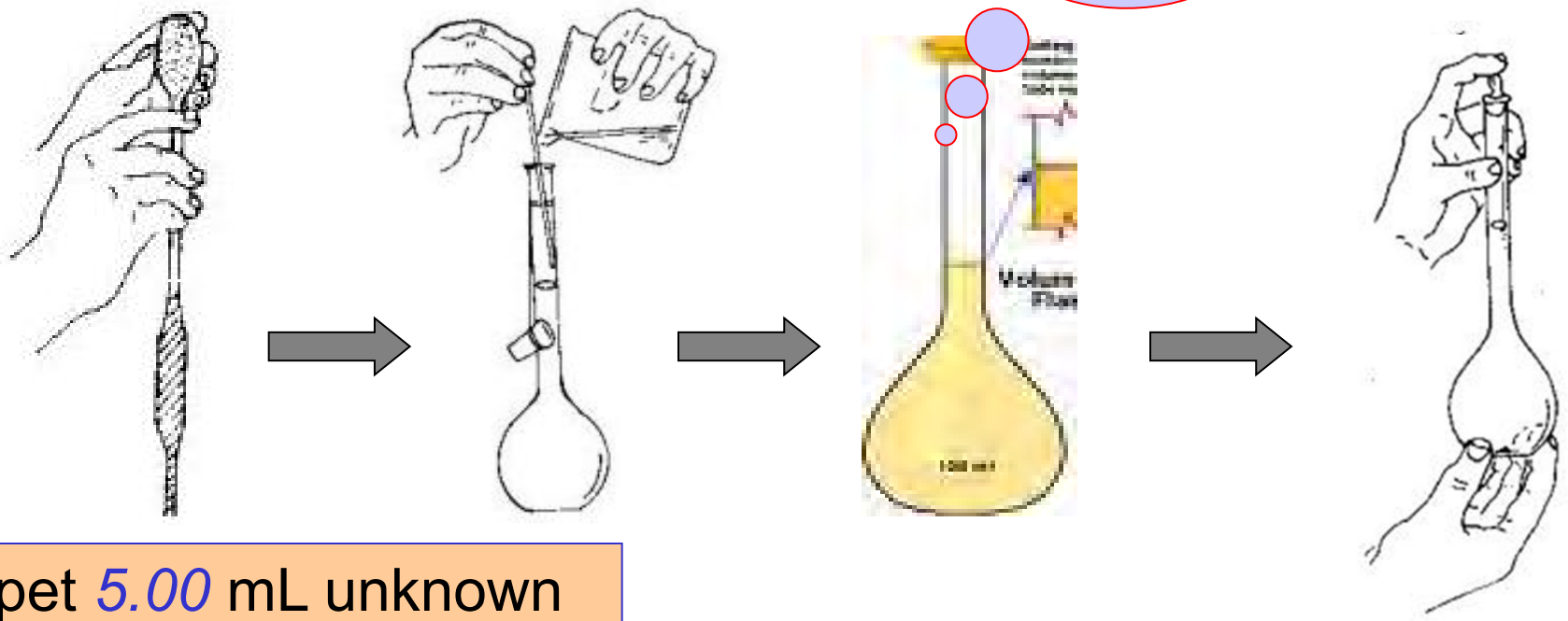
b. Dilute the solution by filling the volumetric flask until the meniscus reach the mark.

c. ¾ fill the rinsed cuvette with the unknown solution. Use the spectrometer to measure A.

d. Determination of unknown concentration by using the calibration curve.

Scheme 2

Attention!
Add drop-wise with a pipet!



Pipet *5.00* mL unknown solution

2.00 mL HNO_3 and *1.00*mL AV solution

Dilute the solution to 50.00mL

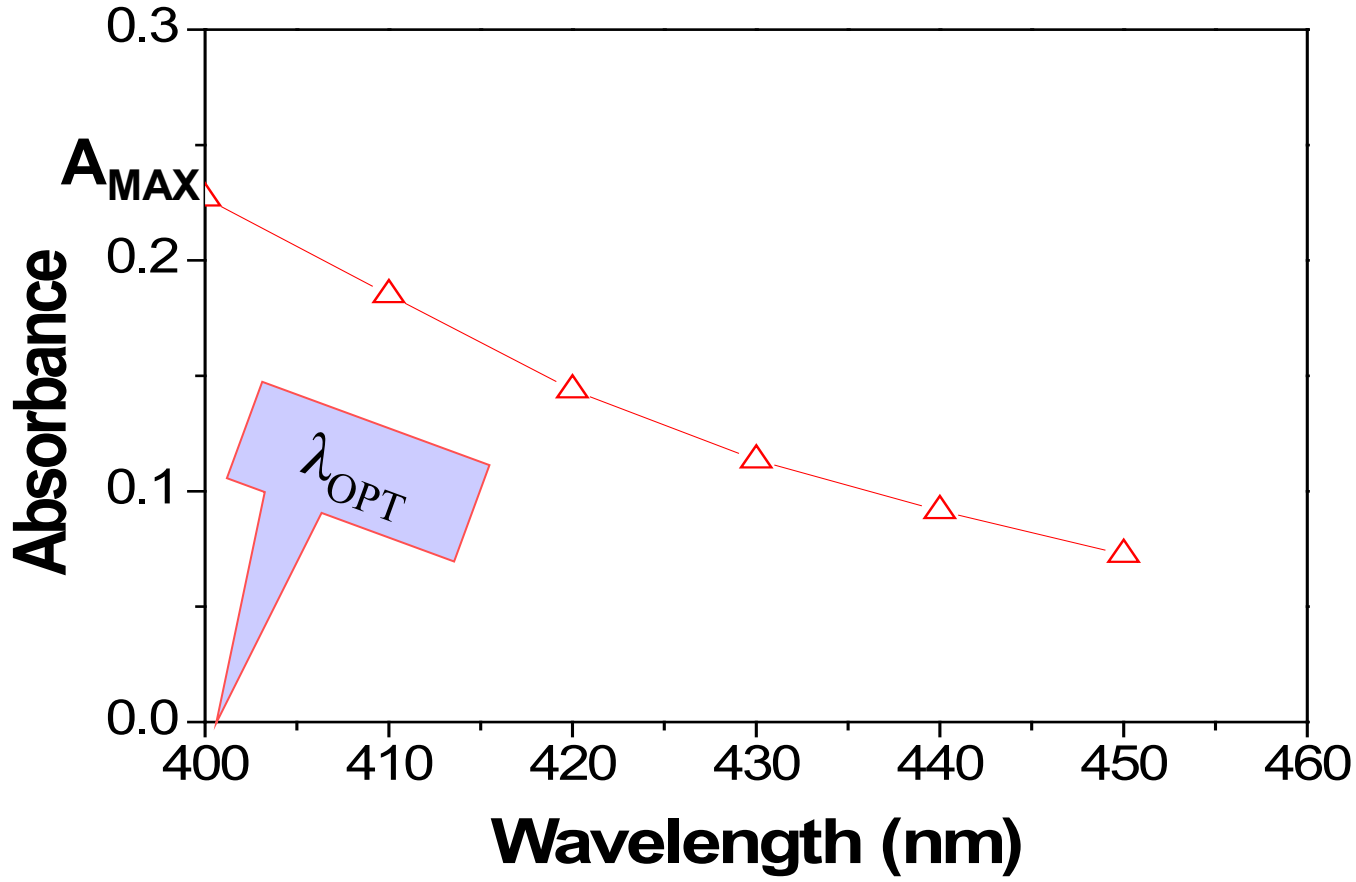
Invert >20 times

E3 INTERPRETING SPECTROMETER ACCURACY

ABSORBANCE VS. WAVELENGTH CALIBRATION CURVE (finding optimum wavelength, λ_{OPT})

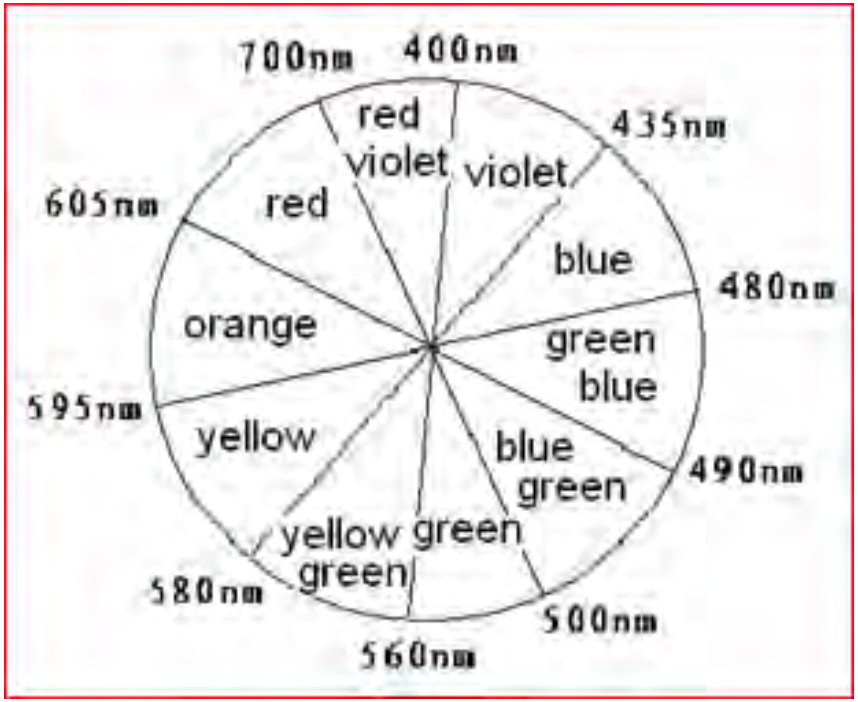
- Accuracy of spectrometer wavelength calibration depends on the light source sensitivity such as using Gas-Discharge Emission lamps.
- For best results, choose a lamp source or combination of sources that matches the analytical wavelengths of interest and spans your measurement range. With more emission lines to utilize, correcting for spectrometer baseline drift and related phenomena inherent to all spectrometers. Be careful interpreting the optimum wavelength.
- The spectrometers used for finding the optimum wavelength are not perfect choice for the phosphorous ions absorbance analysis (meter starting wavelength is 400nm, which is near the maximum absorbance range for phosphorous ions).
- Many factors affect the curve such as experimental errors, working with inaccurate light source, knob dial mechanism, etc. Data can be erratic near the ends of the calibration curve of **A vs. λ_{OPT}** (**λ_{OPT} = 400nm** for our lab spectrometers).
- Another important factor is that the sensitivity of absorbance is dampened (taken the log) when compared with concentration c (**$A = -\log (I/I_0) = \varepsilon bc$**)

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

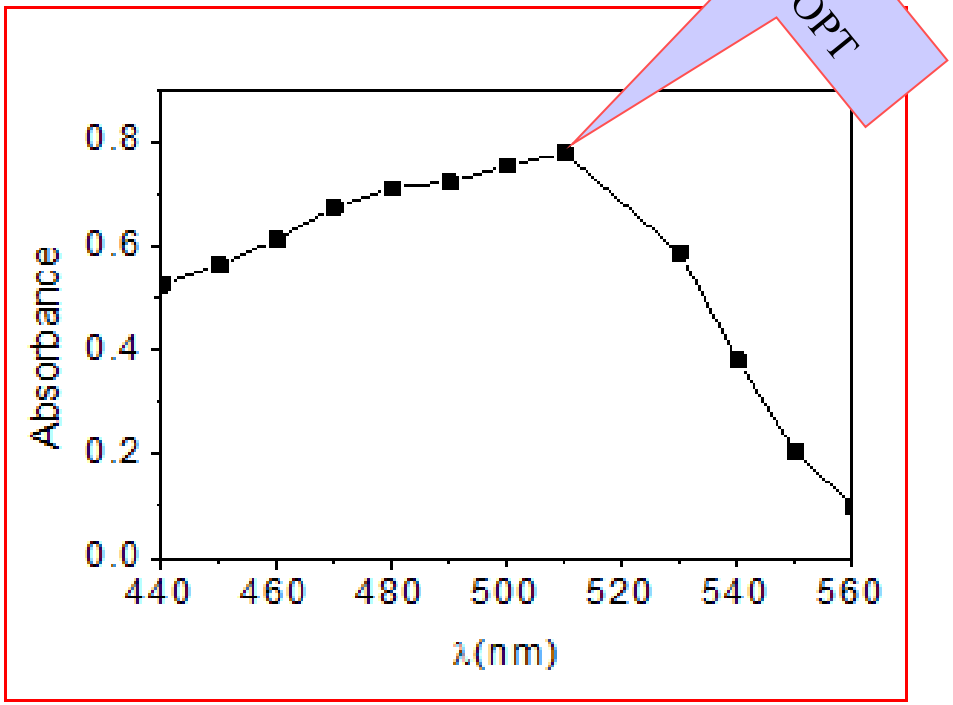


THE ABSORBANCE SPECTRUM

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).

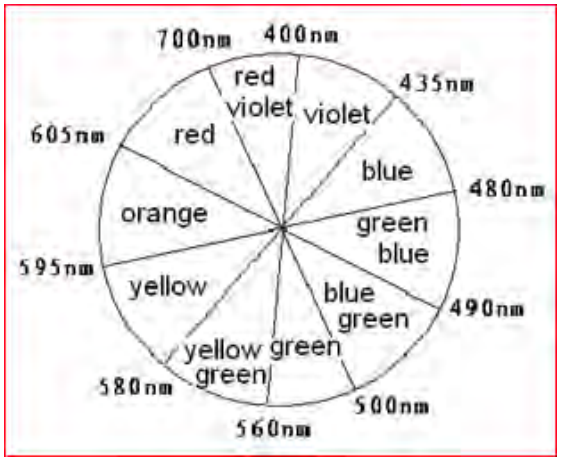
THE ABSORBANCE SPECTRUM

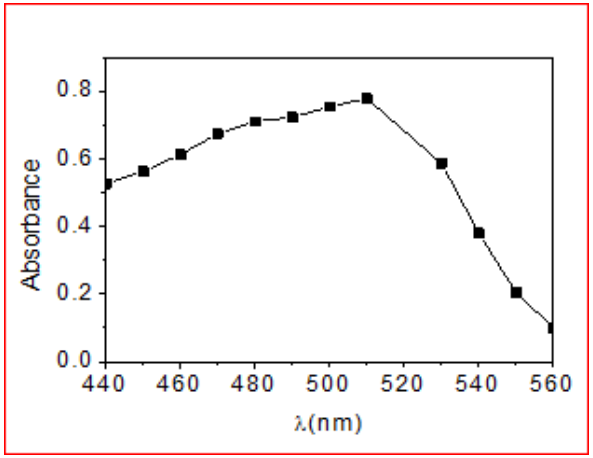
1. Explain % T & A: A = -Log (%T/100%)
2. Graph A & % T, inverted
3. Absorbed vs Observed colors.

VISIBLE COLOR SPECTRUM WITH LIGHT WAVELENGTH

ION ABSORBANCE CURVE: FIND MAX. WAVELENGTH

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

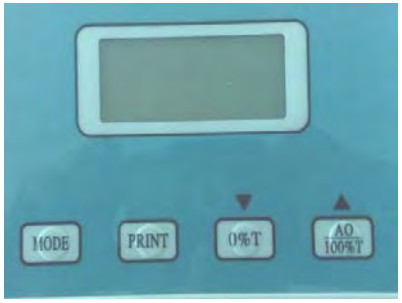


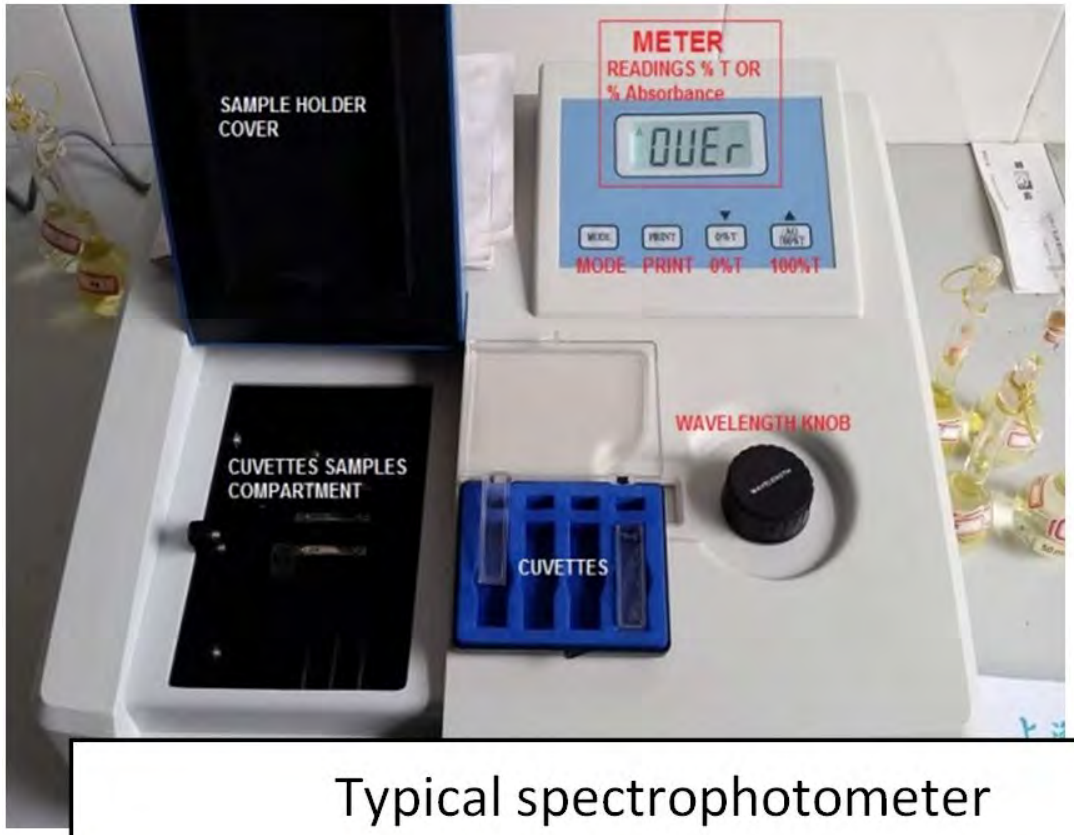


According the complimentary color theory, the color of absorbed light at 520 nm belongs to the GREEN region (which is absorbed), so the color of M^{+n} solution is RED VIOLET (which is reflected into our eyes & see).

Notes on Setting Up the Spectrometer

- **Check that the instrument is turned on. You will hear the fan and see light coming out of the right side of the instrument.**
- **Press the "MODE" button to select Transmittance mode.**





Typical spectrophotometer

Notes on Setting Up the Spectrometer

- **Adjust the wavelength to 400 nm during the initial calibration.**
- Always fill a cuvette (3/4 full) with your blank solvent (Sample labeled 1#) and dry the outside of the cuvette carefully (make sure cuvette is first pre-rinsed 3 times with few mL of blank solvent.
- Insert black block, the blank cuvette Sample #1), and the cuvette of Sample #6 in the first compartment, 2nd and 3rd compartments consecutively and close the cover.
- Press 0%T to set the Transmittance of the black block if the display is different than 000.0.
- Press 100%T to set the **Transmittance** of the blank to only if the display is different than 100.0.

Notes on Setting Up the Spectrometer

- When measuring the samples absorbance make sure you switch the “MODE” button to select **Absorbance mode.** The absorbance value of the sample will now be shown on the LCD.

Important Notes: 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.