(7) FLAME PHOTOMETTTER

Flame photometry more properly called flame atomic emission spectrometry is a relatively old instrumental analysis method. Its origins date back to Bunsen's flame-color tests for the qualitative identification of select metallic elements. Probably the most common example of the atomic emission effect is fireworks.

As an analytical method, atomic emission is a fast, simple, and sensitive method for the determination of trace metal ions in solution. Flame photometry is good only for elements that are easily excited and do not require very high temperatures (Na, K, Li, Ca are the most widely determined atoms by this technique).

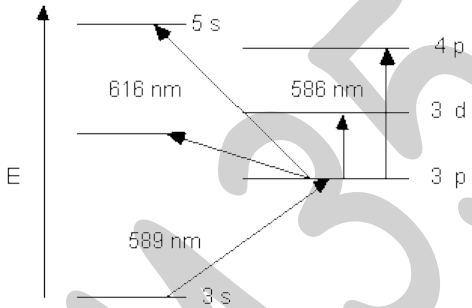
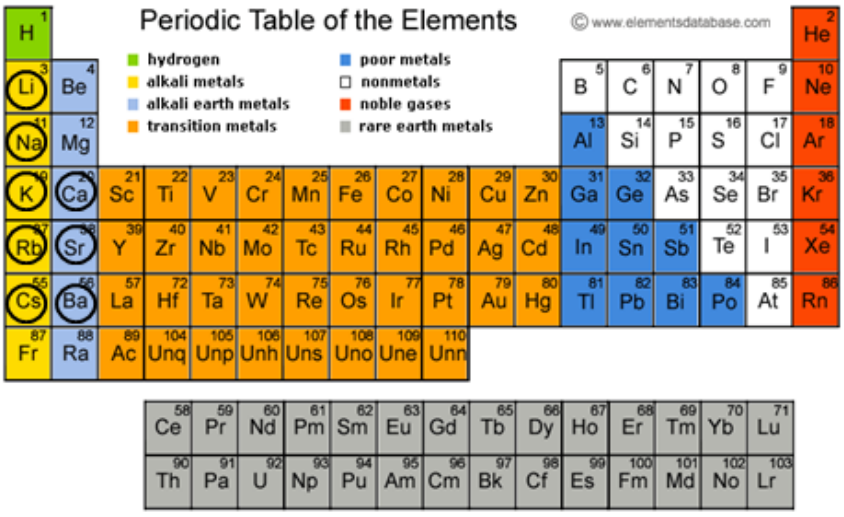


Figure 1: Energy level diagram for atomic sodium (Only few lines are shown)

Detection limits can be quite low. "Good" elements typically have detection limits between about 1 ng/ml and 1 µg/ml.

The method is suitable for many metallic elements, especially for those metals that are easily excited to higher energy levels at the relatively cool temperatures of some flames.



Metalloids and nonmetals generally do not produce isolated neutral atoms in a flame, but mostly as polyatomic radicals and ions. Therefore, nonmetallic elements are not suitable for determination by flame emission spectroscopy, except for a very few and under very specialized conditions.

Flame photometry is a highly empirical, rather than an absolute, method of analysis such as gravimetric tests. That is, you must calibrate the method carefully and frequently. Many different experimental variables affect the intensity of light emitted from the flame and that finding its way to the detector. Therefore, careful and frequent calibration is required for good results.

The instrument is called a "single-channel" photometer because it can determine only one element at a time and has a single direct-reading output.

Instrumentation

A flame photometer instrument is extremely simple where the sample in solution is aspirated through an aspirator or nebulizer into the flame which is usually a propane / air fuel or, even, a purified natural gas/air mixture. The sample matrix evaporates followed by atomization of the sample. Atoms present in the high temperature zone of the flame are excited to higher energy levels by absorbing energy from the flame. As excited atoms return to the ground state they emit radiation in definite wavelength depending on the energy level from which each atom drop. This gives rise to a line spectrum. However, in flame photometry a pre-selected filter (depending on the atom in question) is used and it is the intensity of the emission line that is practically measured and is related to the original concentration of the sample in solution. The detector is usually a phototube or a photomultiphier tube depending on the quality of the instrument. A schematic diagram of a simple flame photometer is shown is shown in Figure 2.

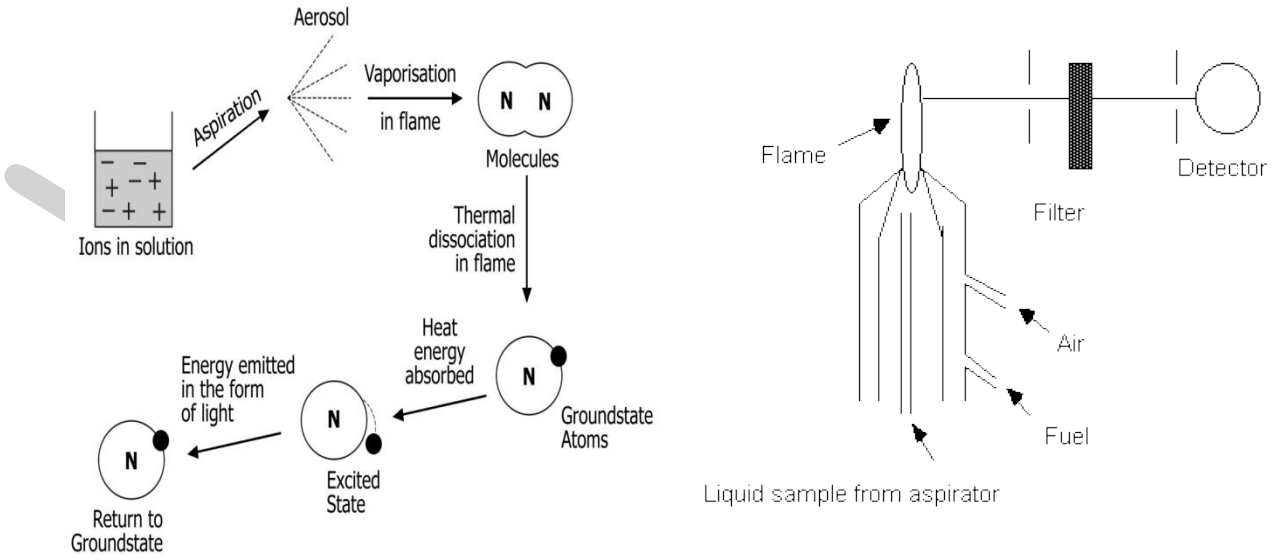


Figure 2: A schematic of a simple flame photometer instrument.

The wavelength of the light emitted from the flame is characteristic of the particular element

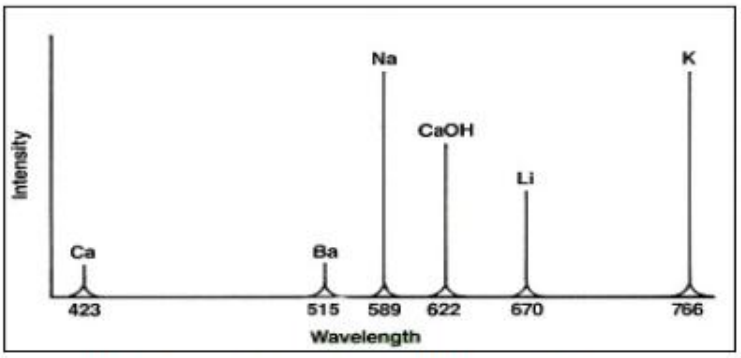


Fig. 2. Intensities of emissions of the elements at equal concentrations and their wavelengths.

The intensity of this light is, in most cases, proportional to the absolute quantity of the species present in the flame at any moment, i.e. the number of atoms returning to the ground state is proportional to the number of atoms excited, i.e. the concentration of the sample. N.B. This relationship applies only at low concentrations.

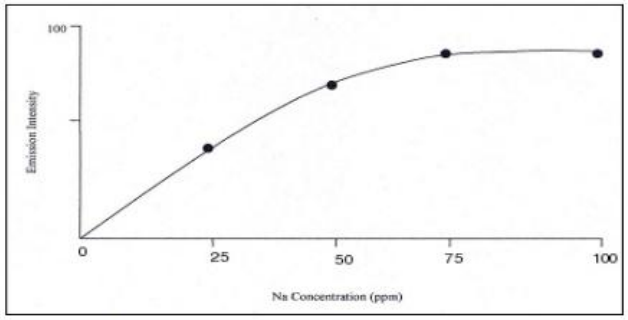


Fig. 3. An example of a calibration curve for an industrial measurement of Sodium concentration.

The emitted radiation is isolated by an optical filter and then converted to an electrical signal by the photo detector

A simple flame photometer consists of the following basic components:

1. A flame that can be maintained in a constant form and at a constant temperature:- "The urner" (see Fig. 4)

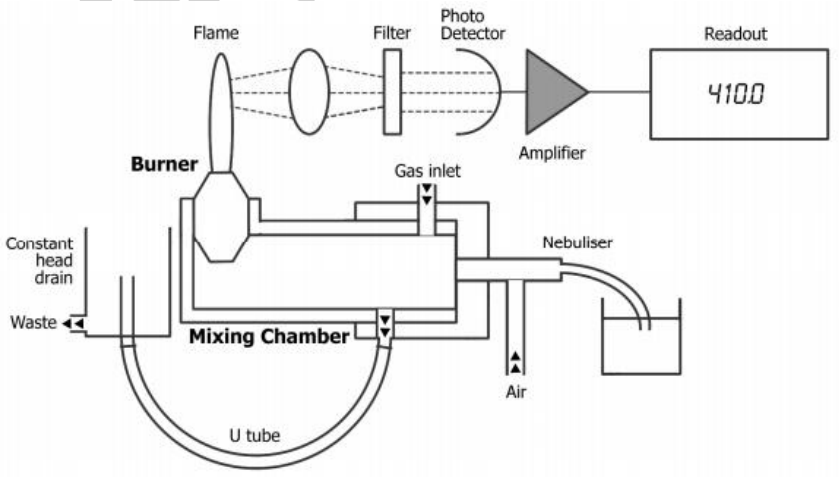


Fig. 4. Basic components

2. A means of transporting an homogeneous solution into the flame at a steady rate:- "Nebuliser and mixing chamber" (see Fig. 4)
3. A means of isolating light of the wavelength to be measured from that of extraneous emissions:-

"Simple colour filters" (interference type) (see Fig. 4)

14. A means of measuring the intensity of radiation emitted by the flame:- "Photo Detector" (see Fig. 4).

Flame Photometer Operating Data

The analysis of alkali and alkaline earth metals by flame photometry has two major advantages.

1. Their atoms reach the excited state at a temperature lower than that at which most other elements are excited.
2. Their characteristic wavelengths are easily isolated from those of most other elements due to wide spectral separation.

The main emission peak for Mg is outside the visible spectrum.

Table 1 shows the temperature achieved by a variety of gas mixtures:

Mixture	Temperature (0C)
Acetylene/Oxygen	3,100 - 3,200
Acetylene/Nitrous Oxide	2,900 - 3,000
Natural gas/Oxygen	2,700 - 2,800
Hydrogen/Oxygen	2,500 - 2,700
Acetylene/Air	2,100 - 2,400
Hydrogen/Air	2,000 - 2,100
Propane/Air	1,900 - 2,000
Butane/Air	1,300 - 1,900
Natural Gas/Air	1,700 - 1,800

Table 1. Temperatures achieved by a variety of gas mixtures

The analysis of Na, K, Li, Ba and Ca are typically determined at low temperatures, i.e. 1500-2000°C, therefore suitable mixtures are propane/air, butane/air and natural gas/ air.

Characteristic Wavelengths of the Elements

It is common knowledge that when sodium is introduced into a flame it emits a radiation in the yellow region of the visible spectrum. Table 2 gives details of the measurable atomic flame emissions of the alkali and alkaline earth metals in terms of the emission wavelengths and the colors produced.

Element	Emission Wavelength (nm)	Flame Colour
Barium (Ba)	515 *	Lime Green
Calcium (Ca)	622 **	Orange
Lithium (Li)	670	Red (Carmine)
Potassium (K)	766	Violet
Sodium (Na)	589	Yellow

Table 2

Table 2 Notes:
* Barium is measured at 515nm to avoid interference with the Ca band at 554nm.
** Calcium is measured by using the Calcium hydroxide band emission at 622nm. However, the main atomic emission occurs at 423 nm.

Preparing for Analysis

1. Avoid handling samples with fingers. This leads to serious contamination.

2. Standards should be stored in sealed vessels and in high concentrations, i.e. store the standards as a stock 1000 ppm solution and prepare dilutions when required. The long term storage of low concentration standards is not recommended *due to degradation of the ionic species*.

3. All analyses involve the use of a diluent which is almost always deionised water. This should be of the highest quality for accurate flame analysis. Sodium, potassium and calcium are present in high concentrations in tap water and thus efficient deionisation is essential if any of the common flame analytes are to be determined.

4. Species that cause interference should be removed from samples or the equivalent concentration of interferent should be present in the standards so as to avoid erroneous results, e.g. if a sample of approximately 10 ppm Na contains approximately 1000 ppm Ca^{2+} then Na^{+} analysis can only be achieved by removing Ca^{2+} using oxalate/oxalic acid or by ensuring all standards contain 1000 ppm Ca^{2+} Always follow a well documented analytical procedure, which should contain information pertaining to interference removal when applicable.

If a finger is immersed in 20 ml of deionised water the resulting Na' concentration will exceed that of a 10 ppm standard.

5. Standards and samples should not be exposed to the atmosphere for long periods due to contamination from airborne particles and the evaporation of the solvent leading to elevated concentrations.
When in doubt about equipment or application the operator should contact the manufacturer for advice.

Sample Preparation

The sample, if not already a solution, must be converted to a media which is suitable for direct introduction into the flame photometer i.e. the sample should be aqueous with no solid matter present.
This is achieved by:
1. Extracting the salts from solid samples using deionised water or suitable extractants, e.g. saturated $CaSO_4$ for sodium in soil. Extraction is made more successfully using a blender macerator or shaking machine.

2. If the sample is organic then the organic material should be removed by ashing. The remaining oxides are then dissolved using strong acids.

3. Filtration/centrifugation to remove solid debris.

When aqueous, the sample can then be diluted to a known, accurately measured volume using deionised water. If it is a concentrated sample then the dilution ratio should be increased. If the sample concentration is low then a small volume of diluent and initial extractant should be used 3.

Standard Preparation

When preparing standards always observe the following:

1. Standards must always contain the constituents that are present in the samples in the same concentration ratios (element being sensed not included), i.e. if samples are prepared in 0.05M HCl then the standards should also contain 0.05M HCl.

2. Always ensure that the standards encompass the expected range of sample concentrations.

3. Standards should be prepared so as to ensure that the region in which measurements are made coincide with the concentrations that produce the optimum performance from the flame photometer, i.e.: when measuring sodium the top standard is ideally 10 ppm; when measuring potassium the top standard is ideally 10 ppm; when measuring calcium the top standard is ideally 100 ppm; when measuring barium the top standard is ideally 1000 ppm.

4. Four standards should be prepared to enable an accurate calibration plot to be drawn.

NOTE: that the blank used should contain all the constituents of the standard solutions except the element that gives rise to the emission.

Making a Measurement

1. Prepare standard and sample solutions.
2. Power up the flame photometer in accordance with the instruments instruction manual
3. Set blank with the diluent used for sample and standard preparation. This is usually deionised water .
4. Aspirate the top standard and adjust the display to the required reading.
5. Re-check the blank and standard settings.
6. Aspirate the prepared standards in increasing concentrations and record their stable display readings.
7. Plot a graph of display reading (intensity) against standard concentration on linear graph paper
8. Aspirate the unknown solution and record the stable display reading.
9. Determine the sample concentration by interpolation from the calibration plot.
10. Operate the instrument shutdown procedure.

Making a Measurement

Aspiration

All Blank, Standard and sample solutions are introduced into the flame by aspiration. One end of small diameter tubing is placed into the sample cup. The other end of the tubing is attached to an Aspiration Needle. Compressed air is forced through a small orifice and past the tip of the needle. The high velocity of the air stream causes the solution to form an aerosol. This enters the Mixing Chamber where it is mixed with the fuel gas and larger droplets are excluded. The small droplets make their way to the flame undergoing the excitation/emission process.

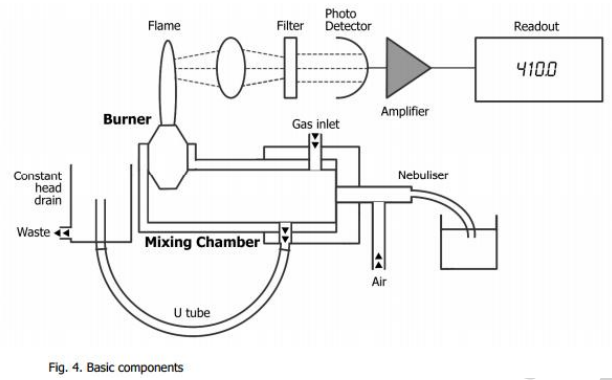


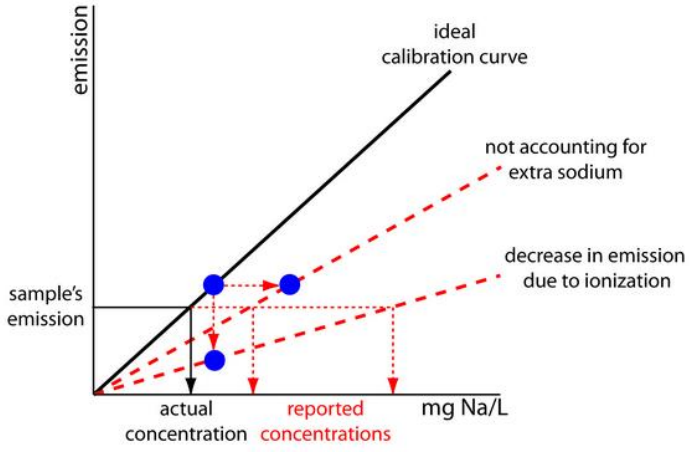
Fig. 4. Basic components

It is important that all solutions be clear of particulates that would block the tubing or needle.

Calibration

Until recently all flame photometry instruments required the operator to do analyses by either of two methods.

1. Stay in the lower concentration ranges (under 50ppm). Calibrate with a Blank and one Standard and read the results directly from the meter or display.
2. To manually graph a calibration curve of a Blank and many Standards. Results are then interpolated from the graph.



The Flame Photometer has simplified the process. When calibrating, the Flame Photometer will prompt the operator to aspirate the Blank and Standards (with the option of one or several Standards) and enter their numerical values. The Flame Photometer electronically monitors for stable readings, stores all data points, develops the calibration curve, and interprets the sample readings.

NOTE: When using clinical flame photometers a calibration curve is not required as the display is calibrated in direct concentration units, therefore only one standard is required to enable this calibration to be performed.

Sample Determination

Once calibrated, sample determination is simply done by aspirating the samples and reading the results from the display

Issues to consider for your practical report

- What are the potential sources of error in this experiment? How could they be overcome?
- Are there alternative methods for determining the concentration of Calcium, Sodium, Potassium and Barium in water? If so, how do they compare to this method?
- What is the source of the River and lake Calcium, Sodium, Potassium and Barium content?
- What are typical water Calcium, Sodium, Potassium and Barium values in sea, rivers, streams, lakes and drinking water? How do your data compare with these values?
- How can Calcium, Sodium, Potassium and Barium be removed for domestic and industrial wastes?
- What are the legal limits (if any) of Calcium, Sodium, Potassium and Barium in drinking water? Do your values exceed such legal limits?
- What are the potential human health and environmental effects (if any) of excess Calcium, Sodium, Potassium and Barium in potable water?

Experiment (7): Flame Photometer
Experimental Results

Name	Date
ID No.	Group

Determination of concentration of unknown solutions:

Sample ID	Dilution Factor of Sample	Standard Concentration ID	Concentration of standard solution (ppm)	Measured Element	Flame photometer Reading (ppm)	Observation (Color and Intensity)

Observations & Calculations:

(8) COLORIMETER

Colorimeter is a light-sensitive instrument that measures how much color is absorbed by an object or substance. It determines color based on the red, blue, and green components of light absorbed by the object or sample, much as the human eye does. When light passes through a medium, part of the light is absorbed, and as a result, there is a decrease in how much of the light reflected by the medium. A colorimeter measures that change so users can analyze the concentration of a particular substance in that medium. The device works on the basis of Beer-Lambert's law, which states that the absorption of light transmitted through a medium is directly proportional to the concentration of the medium.

There are many different types of colorimeters, including the **color densitometer**, which measures the density of primary colors, and the **color photometer**, which measures the reflection and transmission of color. Styles include digital, also called laboratory, and portable. Digital versions are most often used in a lab setting for sampling or in the classroom for educational purposes. Portable versions can be carried anywhere, regardless of environmental conditions, to test things like water and soil samples on site.

The spectrophotometer, a type of photometer that measures light intensity, is often grouped together with colorimeters, but it is technically a different device. Both rely on Beer-Lambert's law to calculate the concentration of a substance in a solution, but they do so in different ways. A colorimeter measures only red, green, and blue colors of light, while a spectrophotometer can measure the intensity of any wavelength of visible light. In general, spectrophotometers are more complicated and less rugged than most colorimeters; they should be handled with utmost care and require regular recalibration.

How a Colorimeter Works

At its most basic, a colorimeter works by passing a specific wavelength of light through a solution, and then measuring the light that comes through on the other side. In most cases, the more concentrated the solution is, the more light will be absorbed, which can be seen in the difference between the light at its origin and after it has passed through the solution. To find the concentration of an unknown sample, several

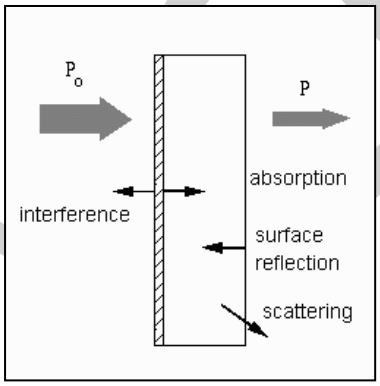
samples of the solution in which the concentration is known are first prepared and tested. These are then plotted on a graph with the concentration at one axis and the absorbance on the other to create a calibration curve; when the unknown sample is tested, the result is compared to the known samples on the curve to determine the concentration. Some types of colorimeters will automatically create a calibration curve based on an initial calibration.

Uses

Colorimetric testing is a widely used method of water testing. A chemical reagent (eg. DPD tablets) is added to a known sample of water. The intensity of colour is then measured to determine the concentration of a particular chemical present in the water (eg. Chlorine). The concentration of the parameter can be determined with a colorimeter, either a Comparator & Disc, (visual system) or a photometer (electronic / digital) system.
Different chemical substances absorb different and varying visual light frequencies. Since the absorption of a substance is proportional to its concentration, i.e. a more concentrated solution gives a higher absorbance reading, therefore; the concentration of a known solute can be measured using a colorimeter.

The Beer-Lambert Law

Many compounds absorb ultraviolet (UV) or visible (Vis.) light. The diagram below shows a beam of monochromatic radiation of radiant power P_0, directed at a sample solution. Absorption takes place and the beam of radiation leaving the sample has radiant power P.



The amount of radiation absorbed may be measured in a number of ways:

$$\textbf{Transmittance, } T = P / P_0$$

$$\textbf{\% Transmittance, } \%T = 100\ T$$

Absorbance,

$$A = \log_{10} P_0 / P$$

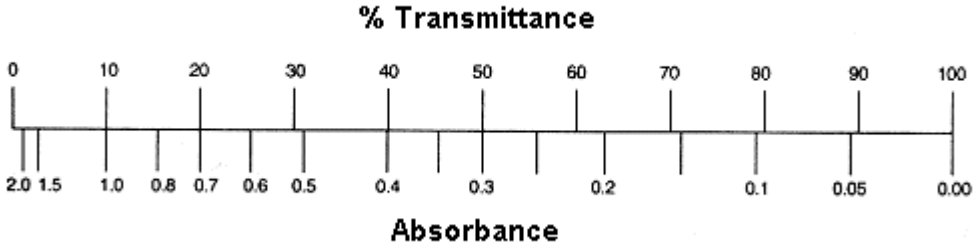
$$A = \log_{10} 1 / T$$

$$A = \log_{10} 100 / \%T$$

$$A = 2 - \log_{10} \%T$$

The last equation **$A = 2 - \log_{10} \%T$** , is worth remembering because it allows you to easily calculate absorbance from percentage transmittance data.

The relationship between absorbance and transmittance is illustrated in the following diagram:



So, if all the light passes through a solution without any absorption, then absorbance is zero, and percent transmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite.

the Beer-Lambert law is:

$$A = \varepsilon bc$$

Where

A is absorbance (no units, since $A = \log_{10} P_0 / P$)

ε is the molar absorbtivity with units of L mol^{-1} cm^{-1}

b is the path length of the sample - that is, the path length of the cuvette in which the sample is contained. We will express this measurement in centimetres.

c is the concentration of the compound in solution, expressed in mol L^{-1}

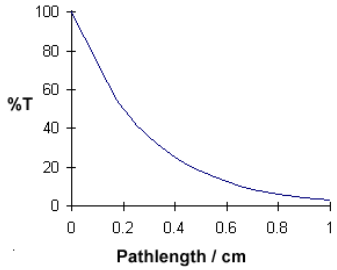
CAUTION: T value is often written as a percent, however, to use the mathematical equation introduced below it must be written as a decimal. (i.e. 0.20, not 20%)

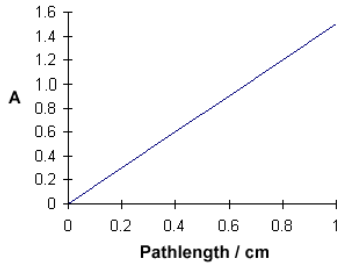
The reason why we prefer to express the law with this equation is because absorbance is directly proportional to the other parameters, as long as the law is obeyed.

$$\%T = 100\ P/P_0 = e^{-\varepsilon bc}$$

Now, suppose we have a solution of copper sulphate (which appears blue because it has an absorption maximum at 600 nm). We look at the way in which the intensity of the light (radiant power) changes as it passes through the solution in a 1 cm cuvette. We will look at the reduction every 0.2 cm as shown in the diagram below. The Law says that the fraction of the light absorbed by each layer of solution is the same. For our illustration, we will suppose that this fraction is 0.5 for each 0.2 cm "layer" and calculate the following data:

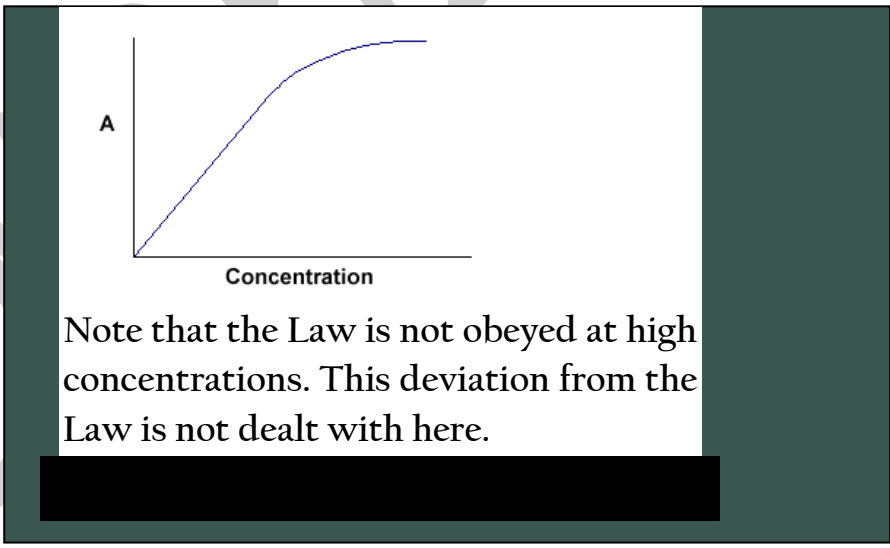
Path length / cm	0	0.2	0.4	0.6	0.8	1.0
%T	100	50	25	12.5	6.25	3.125
Absorbance	0	0.3	0.6	0.9	1.2	1.5





A = εbc tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight line passing through the origin (0,0).

The linear relationship between concentration and absorbance is both simple and straightforward, which is why we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T.



Time required:

Two hour or less depending on the number of samples determined.

Equipment and Reagents:

1. Colorimeter
2. Deionized water washing bottle.
3. Cuvettes.

Significant Experimental Hazards

- Student should be aware of hazards associated with the use of all glassware (cuts).

Procedure:

1. Connect the colorimeter.

2. Look at the solution to be tested and decide which of the four LED wavelengths should be used: 430 nm (violet), 470 nm (blue), 565 nm (green), and 635 nm (red).

Turn the Wheel to select the wavelength.

3. Notice that the cuvettes have ribbed sides and clear sides. Always hold the cuvette by the ribbed sides and insert the clear sides into the light path of the colorimeter indicated by the arrow at the back of the cuvette(slot). Fill cuvettes about ¾ full.

4. Calibrate the device. Insert a cuvette with water (or whatever solvent you are using) into the cuvette slot and close the lid. This will be your blank. Press and hold the CAL button until the red LED starts to flash, then release the button. When the LED stops flashing, calibration is complete.

5. Fill a cuvette with the solution given to you. This will be considered a concentration of 100%. Place in the colorimeter and take a reading.

6. Dilute some of the solution down to 80% this can easily be done in a 10 ml graduated (cylinder). Place in the colorimeter and take a reading.

7. Dilute some of the solution down to 60%. Place in the colorimeter and take a reading.

8. Dilute some of the solution down to 40%. Place in the colorimeter and take a reading.

9. Dilute some of the solution down to 20%. Place in the colorimeter and take a reading.

10. Stop the data collection and run a linear regression curve fit. Record the function.

11. Obtain a sample of the unknown concentration. Return to the Meter screen and insert the unknown sample into the colorimeter. When the reading stabilizes, record the value.

12. Tap the Graph tab and choose Interpolate from the Analyze menu.

Interpolate along the regression curve to determine the concentration of the unknown.

Issues to consider for your practical report

- What are the potential sources of error in this experiment? How could they be overcome?
- Are there alternative methods for determining the color concentration in water? If so, how do they compare to this method?
- How can color be removed for domestic and industrial wastes?
- What are the legal limits (if any) of color in drinking water? Do your values exceed such legal limits?
- What are the potential human health and environmental effects (if any) of excess color in potable water?

There are several ways you can decide which of three wavelengths to use:

Method 1.

Look at the color of the solution. (***REMEMBER THAT THE COLOR OF SOLUTION IS THE COLOR OF LIGHT WHICH IS NOT ABSORBED***).

use a different color of light that will be absorbed

For example: with a blue $CuSO_4$ solution, use the red LED (635 nm).

Method 2.

Place a cuvette containing the solution in the colorimeter.

And check to see which of three wavelengths yields the highest absorbance (low transmittance).

Method 3.

Directions for most colorimetry experiments express a recommended wavelength. Use the closest of the three wavelengths on the colorimeter. Even if the LED wavelength is somewhat different, a Beer's law curve can usually be obtained at almost any wavelength around the recommended

Experiment (8): Colorimeter Experimental Results	
Name	**Date**
ID No.	**Group**

Calibration Curve

Sample ID	Dilution Factor of Sample	Standard Concentration ID	Concentration of standard solution (mg/l)	Absorbance	Transmittance %	Observation (Color and wavelength)

Unknown samples Curve

Sample ID	Dilution Factor of Sample	Standard Concentration ID	Concentration of standard solution (mg/l)	Absorbance	Transmittance %	Observation (Color and wavelength)

Observations & Calculations:

(9) UV-VIS SPECTROPHOTOMETER

A spectrophotometer is a device that can determine "how much color" is in a solution; this can be very useful in determining the concentration of the chemical that gives the solution its color. If a chemical is not itself colored, a dye may be added that bonds to the chemical of interest and forms color in the process. The spectrophotometer works by measuring how much light is transmitted (passed through) a solution. The basic design of a spectrophotometer is shown in Figure 1 (this figure depicts a "colorimeter" a probe that acts as a simple spectrophotometer.) The solution is placed in a cuvette (a square glass or plastic vial) or a test tube, which is then placed into the machine. A light is shone through the cuvette / tube and onto a light detector, which changes the amount of light into an electronic signal. The light must pass through the two opposite smooth sides of the cuvette. Since a colored solution absorbs some of the light, the intensity of the light reaching the detector is lowered, or "absorbed". The amount of light that is absorbed by the solution depends on how many colored molecules are in the path of the light, thus leading to Beer's Law.

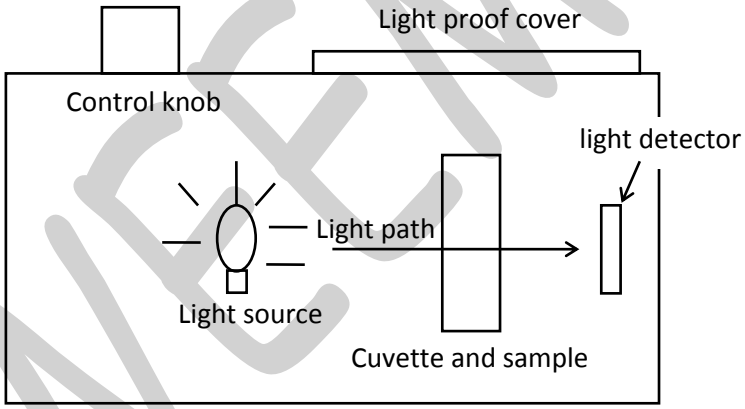


Figure 1a: colorimeter, side view showing inside of machine

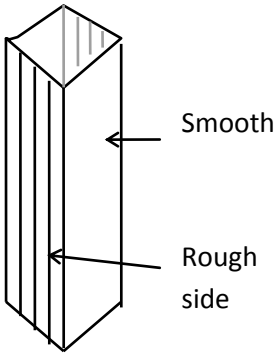


Figure 1b: a cuvette

Single beam and double beam are the two major classes of spectrophotometers. Linear range of absorption and spectral bandwidth measurement are the important features of spectrophotometers.

In Single Beam Spectrophotometers, all the light passes through the sample. To measure the intensity of the incident light the sample must be removed so that all the light can pass through. This type of spectrometer is usually less expensive and less complicated. The single beam instruments are optically simpler and more compact, and can also have a larger dynamic range.

In a Double Beam Spectrophotometer, before it reaches the sample, the light source is split into two separate beams. One beam passes through the sample and the second one is used for reference. This gives an advantage because the reference reading and sample reading can take place at the same time.

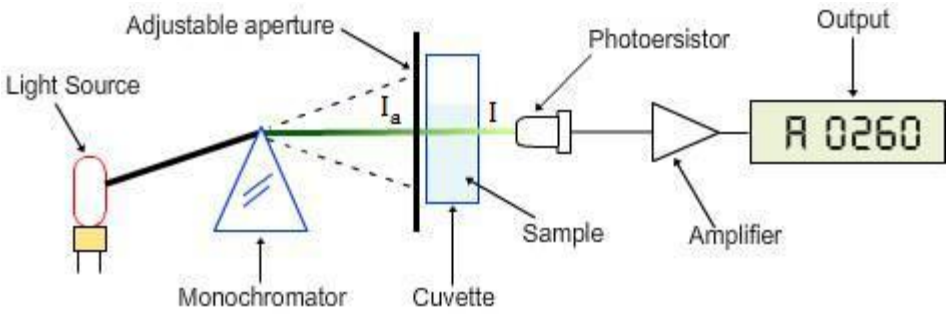
In transmission measurements, the spectrophotometer quantitatively compares the amount of light passing through the reference and test sample. For reflectance, it compares the amount of light reflecting from the test and reference sample solutions.

Many spectrophotometers must be calibrated before they start to analyse the sample and the procedure for calibrating spectrophotometer is known as "zeroing." Calibration is done by using the reference substance, and the absorbencies of all other substances are measured relative to the reference substance. % transmissivity (the amount of light transmitted through the substance relative to the initial substance) is displayed on the spectrophotometer.

The major sequence of events in spectrophotometry is as follows:

1. The light source shines through a monochromator.
2. An output wavelength is selected and beamed at the sample.
3. A fraction of the monochromatic light is transmitted through the sample and to the photo-detector.

Single Beam Spectrophotometer:



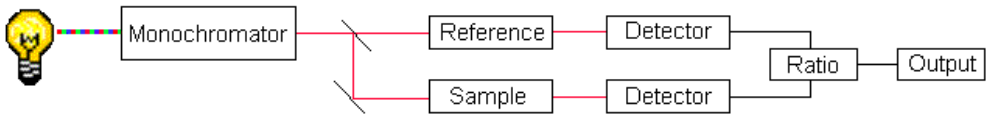
Spectrophotometry deals with visible light, near UV and near IR. To acquire the spectral information quicker in IR spectrophotometers, which use a Fourier transform technique and is called Fourier Transform Infrared (FTIR).

Different Types of Spectrophotometers:

Single Beam: In this type, all the light passes through the sample .To measure the intensity of the incident light the sample must be removed so that all the light can pass through. This type of spectrometer is usually less expensive and less complicated.

Double Beam: In this type, before it reaches the sample, the light source is split into two separate beams. From these one passes through the sample and second one is

used for reference. This gives an advantage because the reference reading and sample reading can take place at the same time.



Schematic diagram of a double-beam UV-Vis. spectrophotometer

Visible Light (400-700 nm): Visible spectrophotometers can use incandescent, halogen, LED, or a combination of these sources and these spectrophotometers vary in accuracy. Plastic and glass cuvettes can be used for visible light spectroscopy.

Ultraviolet Light: UV spectroscopy is used for fluids, and even solids. Cuvettes, only made of quartz, are used for placing the samples.

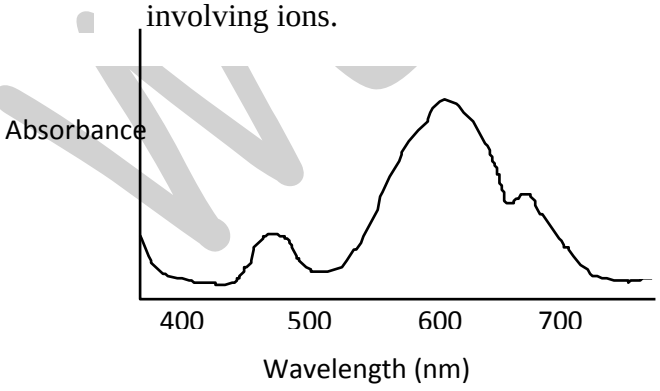
Instruments for measuring the absorption of U.V. or visible radiation are made up of the following components;

Sources (UV and visible)
Wavelength selector (monochromator)
Sample containers
Detector
Signal processor and readout

Infrared Light: IR spectroscopy helps to study different structures of molecules and their vibrations. Different chemical structures vibrate in different ways due to variation of energy associated with each wave length. For example, mid-range and near infrared (higher energy) infrared tends to cause rotational vibrations and harmonic vibrations respectively.

Applications of a Spectrophotometer:

1. It is directly used to measure light intensity at different wavelengths.
2. It is used to determine the unknown concentration of solution.
3. Spectrometers can be used to determine the equilibrium constant of a reaction involving ions.



For the absorbance spectrum graphed here, the best wavelength of light to use is about 605 nm because that is the point of maximum absorbance

Beer's law is a useful relationship for finding the concentration of an unknown solution. It is based on the fact that more concentrated solutions will absorb more light because the light must pass through more molecules in moving through the solution. Mathematically, it states that the concentration (C) of a solution and another property called the absorbance (A) are directly related.

Beer's law: $A = k \cdot C$

Absorbance = constant • solution concentration

Absorbance has something to do with the amount of light that is absorbed. The constant will depend on such things as

1) the nature of the solvent,
2) the wavelength of light used, and
3) the distance the light must travel through the tube or cuvette. These variables are kept constant so that the concentration can be studied. Typically a series of solutions of known concentration (standards) are set up. The absorbance of each is measured and a linear graph is plotted. If the absorbance of any test solution is measured, the graph can be used to find the solution's concentration.

Beer's Law and the spectrophotometer:

The "transmittance" of light mentioned above is easy to understand... it is the fraction of light intensity that passes through the solution. Mathematically, it can be expressed as

$$T = I_t / I_0$$

Absorbance cannot be expressed so simply. The origin of absorbance was learned in our study of electrons. Light is absorbed when it is the right wavelength to excite electrons in molecules in the solution to a higher state. Not all molecules can absorb all wavelengths of light. This is why, the amount of light absorbed (as described by the constant, k, in Beer's law) depends on the wavelength of the light and the solvent. The total amount of absorbance will also depend on the number of molecules the light must pass through. Since the cuvettes in an experiment are all the same width, the distance the light must travel through the solution is controlled, leaving only the concentration of the solution as a variable which increases the interaction of the light with particles. Twice the concentration should make the light contact twice the number of molecules which should yield twice the absorbance, giving rise to Beer's law ($A = k \cdot C$)

Actually, the absorbance of the solution CANNOT be measured directly. A quantity called "transmittance" is measured and mathematically converted to absorbance.

Mathematically, absorbance (A) is NOT the fraction of light absorbed by the solution. Instead it must be related to transmittance. The actual expression uses logarithms:

A = log(1/T) = -log(T)

T must be a number between 0 to 1. The Spectrophotometer works well only in a certain range. The transmittance should be at least 0.28; this means very concentrated solutions with very low transmittance will not work well.

Why is the conversion from T to A necessary?
Recall that Beer's law states that the direct relationship involved is between absorbance and concentration. However, a colorimeter or spectrophotometer can only read transmittance, not absorbance. Thus the transmittance read by the machine must be converted to absorbance to make the plot.

Instrumental components

Sources of UV radiation

It is important that the power of the radiation source does not change abruptly over it's wavelength range.

The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. The mechanism for this involves formation of an excited molecular species, which breaks up to give two atomic species and an ultraviolet photon. This can be shown as;

$$D_2 + \text{electrical energy} \rightarrow D_2^* \rightarrow D' + D'' + h\nu$$

Both deuterium and hydrogen lamps emit radiation in the range 160 - 375 nm. Quartz windows must be used in these lamps, and quartz cuvettes must be used, because glass absorbs radiation of wavelengths
less than 350 nm.

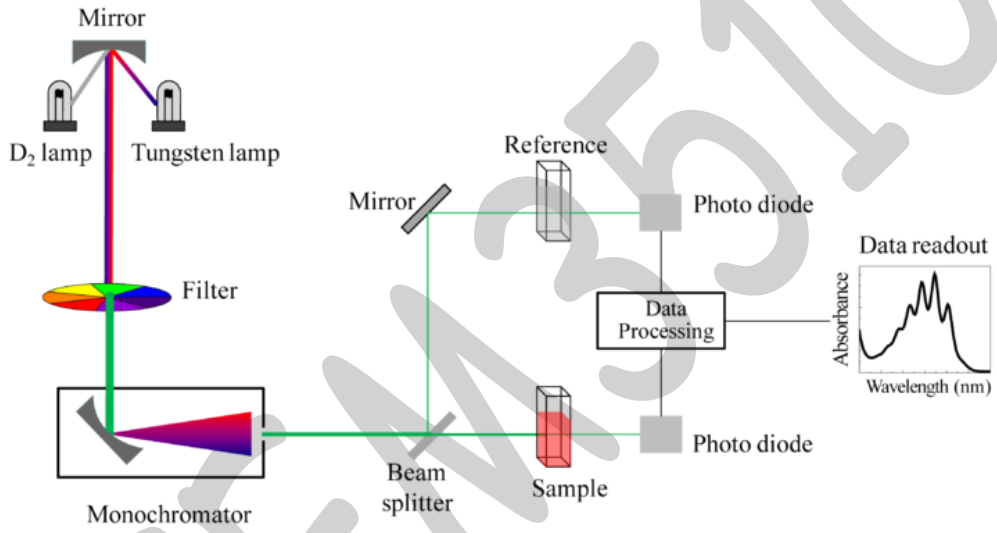
Sources of visible radiation

The tungsten filament lamp is commonly employed as a source of visible light. This type of lamp is used in the wavelength range of 350 - 2500 nm. The energy emitted by a tungsten filament lamp is proportional to the fourth power of the operating voltage. This means that for the energy output to be stable, the voltage to the lamp must be very stable indeed. Electronic voltage regulators or constant-voltage transformers are used to ensure this stability.

Tungsten/halogen lamps contain a small amount of iodine in a quartz "envelope" which also contains the tungsten filament. The iodine reacts with gaseous tungsten,

formed by sublimation, producing the volatile compound WI2. When molecules of WI2 hit the filament they decompose, redepositing tungsten back on the filament. The lifetime of a tungsten/halogen lamp is approximately double that of an ordinary tungsten filament lamp. Tungsten/halogen lamps are very efficient, and their output extends well into the ultra-violet. They are used in many modern spectrophotometers.

Polychromatic radiation (radiation of more than one wavelength) enters the monochromator through the entrance slit. The beam is collimated, and then strikes the dispersing element at an angle. The beam is split into its component wavelengths by the grating or prism. By moving the dispersing element or the exit slit, radiation of only a particular wavelength leaves the monochromator through the exit slit.



One important application is its use in determining the phosphate content of natural and wastewater sources. Phosphate is considered to be one the most important nutrients in natural water. Although several other nutrients (e.g. carbon, nitrogen, sulfur, potassium, calcium and magnesium) are required to facilitate growth of plant material, particularly algae, the phosphorus content is critical in determining the level of algal growth that the water will support. The growth of algae in natural water will rarely occur at phosphate concentrations below 0.05 mg/dm^3. Drinking water may have a maximum allowable phosphate content of 0.3 mg/dm^3, while on average, raw sewage contains about 30 mg/dm^3.

The phosphate found in natural waters mainly exists as the orthophosphate species, PO_4^{3-}, however, the polyphosphates $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$ are frequently encountered. These polyphosphate species may be hydrolysed to produce

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured.

the orthophosphate, however, the species which dominates will depend on the pH prevailing in the particular environment.

Phosphate will readily react with ammonium molybdate in the presence of suitable reducing agents to form a blue coloured complex, the intensity of which is directly proportional to the concentration of phosphate in the solution. The phosphate content of an unknown water sample can be obtained by first plotting the absorbances of a series of standard solutions against the corresponding concentrations, thus giving a calibration curve. The concentration of phosphate in the unknown sample can then be determined from the graph.

Time required:

Two hour or less depending on the number of samples determined.

Equipment and Reagents:

Spectrophotometer
Deionized water washing bottle.
Quartz Cuvettes.
Volumetric flasks
Pipette
Stock solution of phosphorus
Combined reagent

Significant Experimental Hazards

- Student should be aware of hazards associated with the use of all glassware (cuts).
- Student should be aware of hazards associated with the use of all Chemicals used in this test

Procedure:

Preparation of Calibration Curve

1. Prepare a standard stock solution of phosphorus of approximately 100 mg P/dm3 by dissolving 0.11g of KH_2PO_4 (this should be accurately weighed) in distilled water and diluting to 250 ml in a volumetric flask (Stock Solution A).

2. Accurately transfer 10 ml of this solution to a 250 ml volumetric flask (Stock solution B) and make up to volume with distilled water.

3. Use stock solution B to prepare standards of approximately 0.20, 0.40, 0.60, 0.80 and 1.0 mg P/l, that is, pipette 5, 10, 15, 20 and 25 ml portions respectively to separate labeled 100 ml volumetric flasks.

4. Place roughly 50 ml of distilled water into a 100 ml flask as a blank solution, then organize all the analytical solutions for colour development.

Analysis of Water Sample

5. A water sample that has been diluted by a factor of 10 will be provided.

6. Pipette duplicate 25.0 ml portions of the diluted sample to two separate 100 ml volumetric flask, then develop the colour as outlined below.

DO NOT MAKE UP THE SOLUTIONS TO THE MARK YET.

Colour Development

7. Add distilled water to all the analytical solutions (standards and samples) so that each flask contains roughly 50 ml of solution. Starting with standard 1, add 13 ml of COMBINED REAGENT using a 25 ml measuring cylinder .

8. Shake thoroughly and make up to the mark with distilled water.

9. Treat all the solutions similarly then allow 30 minutes for colour development.

Prior to measurement

10. Set the wavelength of the instrument to 880 nm then zero with distilled water in a 1-cm cuvette.

11. Measure the absorbances of the standards in order of increasing concentration followed by that of the sample solutions.

Record your results in the table provided then plot a graph of the *corrected* absorbance vs the corresponding concentration.

★★★★★★★★★★

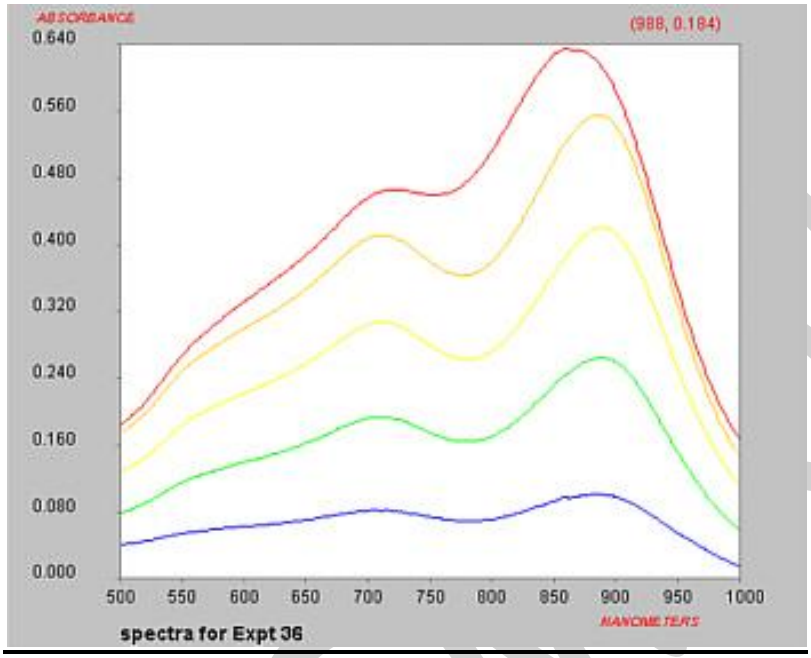
COMBINED REAGENT:

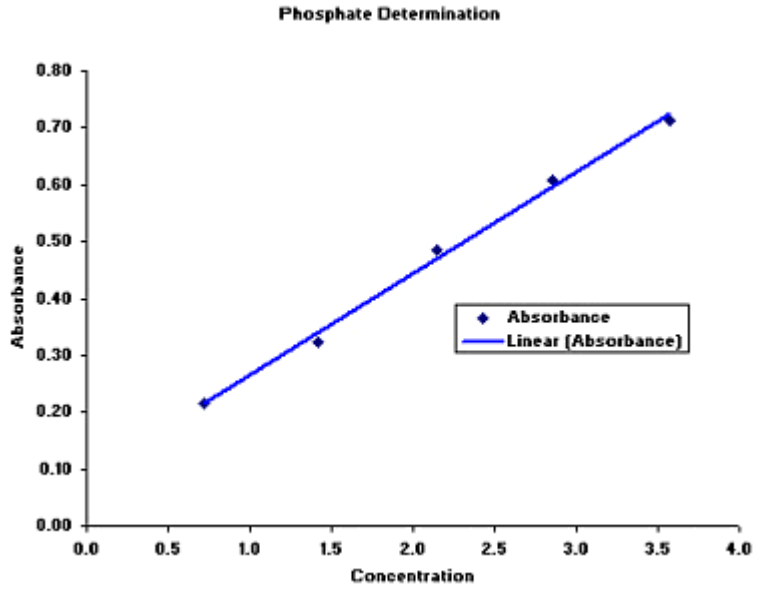
Prepared by combining 500 ml of 2.5 M H_2SO_4, 50 ml potassium antimony tartrate solution (i.e. prepared by dissolving 1.371 g $K(SbO)C_4H_4O_6.0.5\ H_2O$ in about 400 ml distilled water and diluting to 500 ml) and 150 ml ammonium molybdate solution (20 g $(NH_4)6Mo_7O_6.4H_2O$ in 500 ml of water), 300 ml ascorbic acid solution (made by dissolving 5.28 g of ascorbic acid in 300 ml of H_2O). The solution is thoroughly shaken and stored in plastic bottles. A fresh mixture is made on the morning of each lab day.

Issues to consider for your practical report

- What are the potential sources of error in this experiment? How could they be overcome?
- Are there alternative methods for determining the Phosphate? If so, how do they compare to this method?
- How can Phosphate be removed for domestic and industrial wastes?
- What are the legal limits (if any) of Phosphate in drinking water? Do your values exceed such legal limits?
- What are the potential human health and environmental effects (if any) of excess Phosphate in potable water?

Experiment (2): Solids in Water (A) Experimental Results	
Name	**Date**
ID No.	**Group**





Observations & Calculations: