**The Spectrophotometer: Phosphate**

**Objectives**

This exercise introduces you to the principle and use of the spectrophotometer, an analytical instrument used to measure light absorption. You will determine the absorption spectrum of a green food color.

**Introduction**

The spectrum of electromagnetic radiation, of which visible light is a component, is shown in the figure. The visible portion (so called because the human eye can detect it) forms a very small portion of the entire spectrum. Towards the 350 nm end is deep violet, around 500 nm is green, 600 nm is orange, and 700 nm is red. A mixture of all the visible light wavelengths taken together is perceived by the eye as white light.

0.057

Lamp Lens Slit

Lens

Grating

Meter Detector Filter Cuvette Slit

**Wavelength (nm)**

- 200 300 400 500 600 700 800 900 1000 -

x-rays UV Visible Near Infrared

 Infrared

Many chemicals (or substances) absorb light at different wavelengths, and that is why the eye can see an object or material as colored. When white light strikes green glass, chemicals in the glass absorb all the wavelengths *except* for green. With a piece of red glass, all wavelengths *except* red are absorbed.

In this experiment, you are going to use an instrument called the spectrophotometer, to determine the relative amount of light absorbed by a compound at different wavelengths. The essential internals of a spectrophotometer is shown in the figure.



White light emitted by the *lamp* is concentrated by two *lenses*, and focused on a *grating*. The grating splits the white light into the various colors, just like a prism. By changing the angle at which the grating reflects light, the required wavelength is concentrated on the *slit*. The light then passes through the *cuvette*. The cuvette is a glass tube that holds the chemical solution, the absorbance of which you want to measure. The chemical absorbs the wavelength, and the unabsorbed light passes through a *light filter* to the *detector*, which measures the amount of light. The result is displayed on the *meter*.

The controls of the Spectronic 20 are shown in the figure. Please familiarize yourself with the function of each before you start.

With the spectrophotometer, you can determine the light absorption of a chemical at different wavelengths. When the data is graphed, it is called an *absorption spectrum* (figure). In this experiment, you will determine the absorption spectrum of a green dye that is used as a food color.

Wavelength

Absorbance



**Pre-Lab Experiment 10**

1. **Read** the pages covering the theory behind spectrophotometry.
2. **On a separate paper, complete the following:**
* Write the chemical symbol or formula for the following: nitrate \_\_\_\_\_\_\_\_ phosphate \_\_\_\_\_\_\_\_\_

 mercury \_\_\_\_\_\_\_\_\_ chloride \_\_\_\_\_\_\_\_\_\_\_ table salt \_\_\_\_\_\_\_\_\_ lead \_\_\_\_\_\_\_\_\_

* Define the following terms: *SDWA Beer’s Law quantitative qualitative:*

**Materials**

Green food dye Distilled water 2 cuvettes

**Procedure**

1 Turn on spectrophotometer, and let it warm up for 15 min.

1. Set mode to Transmittance.

3 Set wavelength by turning Wavelength Dial.

4 Select the correct filter position (<600 nm - left; >600 nm - right).

5 Set 0% Transmittance using the 0%T knob. *Do not touch this knob until you are back to this step.*

6 Wipe the outside of the cuvette containing distilled water (Blank) with a Kimwipe, and insert into the cuvette holder. *White vertical line must face the front of the instrument.*

1. Switch mode to Absorbance.
2. Rotate 0A knob till the meter reads 0.

9 Remove the Blank cuvette, and insert the Sample cuvette.

10 Record the Absorbance of the solution in the worksheet below and remove the sample cuvette.

11 If you need to take absorbance readings at other wavelengths, change the wavelength, and repeat Steps 2 -10, *after making sure that the filter lever is in the correct position.*

12 After all the readings are complete; plot the absorption spectrum on the graph paper provided.

Note: Fill the cuvette up to the white mark on the cuvette. **Do not overfill.**

 **Spectrophotometer**

**Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Grade:\_\_\_\_\_\_\_\_**

1 Record the absorbance values at each wavelength.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **λ (nm)** | **Abs.** | **λ (nm)** | **Abs.** | **λ (nm)** | **Abs.** | **λ (nm)** | **Abs.** |
| 350 |  | 430 |  | 510 |  | 590 |  |
| 370 |  | 450 |  | 530 |  | 610 |  |
| 390 |  | 470 |  | 550 |  | 630 |  |
| 410 |  | 490 |  | 570 |  | 650 |  |

2 On the graph paper below, mark the (horizontal) **Wavelength** axis in equal intervals from 350 nm to 660 nm.

3 Mark the (vertical) **Absorbance** axis in equal intervals from 0 to a round number above your highest known absorbance (i.e. if the highest absorbance is 0.52, mark it up to 0.6).

4 Plot the absorbance at each wavelength, and draw a smooth curve through the data points.

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|  AbsorbanceWavelength (nm) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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350 400

**Results – 2 peaks with a valley**

Top of highest peak: Wavelength: \_\_\_\_\_ nm Absorbance: \_\_\_\_\_\_\_\_

Top of second highest peak (shorter mountain)Wavelength: \_\_\_\_\_ nm Absorbance: \_\_\_

2 What colors are associated with the two highest peaks? \_\_\_\_\_\_\_\_\_\_ and \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

1. Explain why the green food color has these two peaks/valley.

**Spectrophotometric Estimation of Phosphate**

**Objectives**

In this experiment, you will learn to use the spectrophotometer to perform *quantitative* measurements, by determining the phosphate concentration of an unknown water sample.

**Introduction**

Quantitative spectrophotometry is based on the observation that the greater the concentration of a light-absorbing chemical, the greater is the amount of light absorbed. This relationship is referred to as **Beer's law**, which is formulated as:

Concentration

Absorbance

*At a given wavelength, the amount of light absorbed is directly proportional to the concentration of the light-absorbing substance*.

This relationship is best measured at the absorption maximum, i.e., the wavelength at which light absorption is the greatest. Performing the analysis at this wavelength affords the highest sensitivity for the measurement, *which means that you can distinguish samples that differ even slightly in concentration*.

A practical application of Beer's law is the determination of the concentration of chemicals in a sample, e.g. sodium in water, or calcium in blood. In this experiment, you will estimate the amount of phosphate in drinking water; phosphate is an important environmental pollutant.

Phosphate by itself does not absorb light. However, phosphate reacts with two chemicals, ammonium molybdate and stannous chloride, to yield a compound called molybdenum blue, which is colored. The intensity of the blue color is dependent on phosphate concentration.

In order to determine the exact phosphate concentration of an unknown sample, it is first necessary to define the exact relationship between phosphate concentration and absorbance. This is known as the *standard curve*, and will look like the figure above. Once the curve has been obtained, the phosphate concentration of unknown samples can be determined, by treating them in the same manner in which the standards were treated. Briefly:

**Standards**: Known concentrations Determine absorbance Standard curve

**Unknown**: Determine absorbance From standard curve Find concentration

**Materials:**

Spectrophotometer Water sample 0.1 ppm, 0.5 ppm phosphate standards

10 ml, 1 ml pipets 100 mm cuvettes (x 4) 25 ml Erlenmeyer flasks (x 3)

Test tube rack Pipettor

**Procedure** Fill the cuvette up to the white line. **Do not overfill.**

1 Label three Erlenmeyer flasks as .1, .5, and U in pencil.

2 Measure 5 ml of Standard 1, Standard 2, and the Sample into the corresponding flasks.

3 Add 7 drops of Ammonium Molybdate reagent to each flask, and swirl to mix.

4 Add 1 drops of Stannous chloride reagent (dropper bottle) into each flask, and swirl to mix.

5 Allow the color to develop in the flasks for 10 min.

6 Follow the Spectronic 20 instructions to set up the spectrophotometer at **690 nm**.

7 Set the Absorbance to zero with the Blank (ultra-pure water), and remove.

8 Pour the 0.1 ppm Standard, 0.5 ppm Standard and the unknown (U) sample into their specific cuvettes, insert individually into the spectrophotometer, read the absorbance, and record.

1. After the instructor has reviewed your results, pour the waste into the waste flask, and rinse the cuvettes two times with ultra-pure water.

**Phosphates**

**Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

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| --- | --- | --- |
| **Data** | **Concentration (ppm)** | **Absorbance** |
| Standard #1 | 0.1 |  |
| Standard #2 | 0.5 |  |
| Unknown sample | Xxxxxxxxxxxxxxxxxxx |  |

*Prepare the* ***standard curve*** *for phosphate*

1 Mark the horizontal axis (Concentration) in equal intervals from 0 to 0.5 ppm.

2 Markl the vertical axis (Absorbance) in equal intervals from 0 to a round number above your highest Absorbance.

3 Plot your two known points for Standards 1 and 2. Draw a straight line that extends through these points towards the origin (x=0, y=0). *If you did the experiment right, the line should pass through the origin.*

*Determine the phosphate concentration of the* ***unknown*** *sample*

4 On the Absorbance axis, mark the Absorbance of the *unknown*.

5 From this point, use a straightedge to follow the line **horizontally** till it intersects the graph line.

6 From the intersection point, use the straightedge **vertically** to mark the intersection point on the Concentration axis. This is the phosphate concentration of the unknown.

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| Absorbance |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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Concentration (ppm)

**Results**

The phosphate concentration of the unknown sample is \_\_\_\_\_\_\_\_\_\_\_\_\_\_ ppm.

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| A Local Connection from www.sustaincapecod.org  |
| What are the Most Important Goals?• Informed populace• Visionary leaders• Engaged institutions• Accessible services• Good health• Continuous education• Directed growth• Integrated infrastructure• Valued ecosystemsWhat Can We Do?**Individuals:**• Take shorter showers.• Water gardens and grass at dusk and dawn.• Water gardens with soaker hoses rather than sprinklers.• Plant species that are native and require less supplemental watering.• Install water-saving faucets and shower heads.• Fix dripping faucets.• Flush toilets only as necessary.• Install water-saving toilets.• Utilize town hazardous-waste collections to dispose of paint and other | What is this Indicator?The Drinking Water Quality Indicator tracks the level of nitrate-nitrogen (nitrate) - measured in parts per million (ppm) - in our public drinking water supply. Nitrate is a dissolved form of nitrogen that is commonly found in lawn fertilizers and wastewater effluent. Excessive nitrogen is unhealthy for humans, especially babies, and can cause extensive ecosystem damage in coastal surface waters because it stimulates the growth of algae, which consumes oxygen that aquatic organisms need to survive. Nitrate-nitrogen is also the primary contaminant coming from septic systems, which are the predominant form of wastewater disposal on the Cape. It is important to note that the indicator focuses on the data collected by public water suppliers and small-volume wells, but does not include any data from private wells that are prevalent on the Outer Cape.**Drinking Water Quality**A maximum nitrate-nitrogen concentration of 10 ppm for drinking water supplies has been established by the USEPA and state regulations to avoid impacts on sensitive individuals. Nitrate can limit oxygen in the blood and lead to "blue-baby syndrome." The Cape Cod Regional Policy Plan endorses a nitrogen discharge standard of 5 ppm to ensure that nitrates in drinking water fall well below the federal standard to protect our ponds and coastal embayments from over-enrichment. Nitrate is also an indicator for other contaminants commonly associated with septic system effluent, which may include household chemicals, such as solvents, cleaners, petroleum compounds and pharmaceuticals.  |