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CH 362  
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I pledge my honor that I have abided by the Stevens Honor System.

**1) Title of Experiment:**

Determination of Weight Percentage of Vitamin C in Commercial Vitamin Supplement and Quantitative Analysis of Multi-Component Samples using UV-vis Spectroscopy

Date: October 11, 2020  
Name of Technique: UV-Visible Spectroscopy

**2) Technique:**

UV-Visible spectrometry is similar to other methods of spectrometry, in that it is often used to determine the concentration of an unknown substance by taking advantage of the maximum absorption wavelength of a specific compound. In UV-visible spectrometry, depending on whether the compound absorbs in the visible or the UV spectrum, a compound will be dissolved in water and put in a cuvette of either glass or quartz, respectively. Also, a reference cuvette will also be prepared, that represents a solution with 0 concentration of the unknown compound. Then, both cuvettes are placed into the machine that performs the UV-vis spectrometry. Inside the machine, a monochromator selects certain wavelengths from a light source, and splits it into two beams for the reference and the sample cuvettes. Then, after directing both beams through the cells at varying wavelengths, the detector measures absorbance of the light, and an absorbance curve is recovered over multiple wavelengths, where the maximum absorption wavelength is also identified. Figure 1 outlines the path of the light beam through the device.

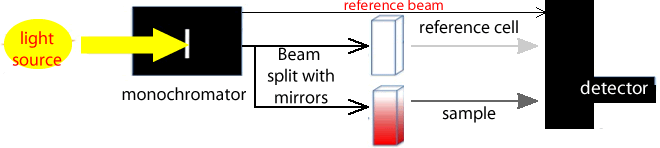


Figure 1: UV-vis Spectrometry Setup

By combining readings of known and unknown concentrations of solutions in the cuvette, Beer’s Law and other calculation methods can be used to determine the concentration of the unknown solution. Figure 2 shows how Beer’s law applies to this experiment.

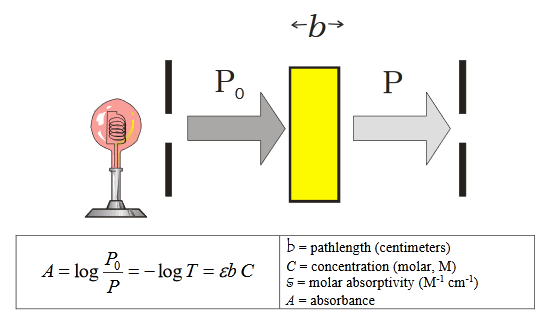


Figure 2: Beer's Law and UV-vis Spectrometry

**3) Application of the Technique to my Experiment:**

In this experiment, UV-vis spectrometry will be used to both measure the weight percentage of vitamin C (ascorbic acid) in a commercial vitamin supplement and determine the concentrations of different compounds in the mixed solution.

In the first part of the experiment, Ordinary Linear Calibration will be used to determine the unknown concentration of a solution of vitamin C. First, a stock solution of ascorbic acid will be prepared, and four solutions of different known concentrations will be prepared using this stock solution. For this experiment, because ascorbic acid absorbs in the UV spectrum, quartz cuvettes will be used. After using ethanol to determine a baseline reading which will calibrate the machine, measurements of each of the four calibration solutions will be taken with the machine by filling the same cuvette with each solution, one at a time. Finally, the vitamin C tablet will be crushed, dissolved, and diluted into a solution to be put in a cuvette and measured. After obtaining all five absorption spectrums, a linear regression at the maximum absorption wavelength can be calculated to determine the concentration of the unknown solution, and the weight percentage of the vitamin C in the tablet can be recovered.

In the second part of the experiment, quantitative analysis on a solution that contains a mixture of cobalt (II), copper (II), and nickel (II) ions will be performed. First, three calibration solutions of each ion individual will be prepared and put in the machine, with deionized water as a reference. Because these ions absorb in the visible light spectrum, a glass cuvette will be used. After obtaining curves of each of the three samples, their maximum absorbance wavelengths will be identified, and the unknown sample will be measured. By determining the absorbance of the unknown at the three maximum absorbance wavelengths, three Beer’s Law equations in a system can be solved to determine the concentration of each ion in the unknown solution.

**4) Calculations:**

**Preparation of stock solution of ascorbic acid**

|  |  |
| --- | --- |
| Mass of ascorbic acid used | 0.010 g |
| Volume of solution | 100.00 mL |
| Concentration of solution (ppm) | 0.010 \* 1000 / 100 / 1000 = 100 ppm |

**Preparation of calibration solutions of ascorbic acid**

ppm of 25 mL solution with x mL of 100 ppm stock solution added:

100 ppm implies 0.1 mg/mL solution added.   
x mL added implies x\*0.1 mg added.   
x\*0.1 mg / (25.00 mL /1000) implies concentration = x/0.25

|  |  |  |  |
| --- | --- | --- | --- |
| Solution Number | Volume of stock solution added (mL) | Volume of solution (mL) | Concentration (ppm) |
| 1 | 1.00 | 25.00 | 4 |
| 2 | 2.00 | 25.00 | 8 |
| 3 | 3.00 | 25.00 | 12 |
| 4 | 4.00 | 25.00 | 16 |

Preparation of 50 mL 0.02 M Cu(II), Co(II), and Ni(II) solutions from 0.05M stock solutions

|  |  |
| --- | --- |
| Concentration of desired solution | 0.02 M |
| Volume of desired solution | 50 mL = 0.050 L |
| Moles of ion needed | 0.02 mol/L\* 0.050 L = 0.001 mol |
| Volume of stock solution | 0.05 M |
| Volume of stock solution needed | 0.001 mol/ 0.05 mol/L = 0.020 L = 20 mL |

**5) References:**

1. Analytical chemistry - UV visible spectroscopy <http://www.dynamicscience.com.au/tester/solutions1/chemistry/analytical%20chem/uvspec.htm> (accessed Oct 11, 2020).
2. Attygalle, A. Instrumental Analysis I Lecture and Laboratory Manual <https://sit.instructure.com/courses/38802/files/6982711?module_item_id=1042514> (accessed Oct 11, 2020).

**6) MSDS:**

**Ascorbic Acid:**

CAS No.: 50-81-7  
Molecular Weight: 176.12  
Chemical Formula: C6H8O6  
Appearance: white solid  
Lab Protective Equipment: Lab coat, goggles  
Strong reducing agent.

**Health effects:**May cause irritation to eyes and skin, and respiratory tract.

**First Aid measures:**Eye contact: rinse immediately with water, especially under eyelids, for >15 minutes.  
Skin contact: wash off immediately with plenty of water and soap.  
Inhalation: Move to fresh air. Get medical attention immediately if symptoms occur. If not breathing, give artificial respiration.  
Ingestion: Rinse mouth with water.

**Other hazards:**Fire: risk if exposed to oxidizing agents.  
Explosion: risk if exposed to oxidizing agents.

**Ethanol:**

CAS No.: 64-17-5  
Molecular Weight: 46.07  
Chemical Formula: C2H6O  
Appearance: colorless liquid  
Lab Protective Equipment: Lab coat, goggles  
Highly flammable liquid and vapor.

**Health effects:**Causes serious eye irritation

**First Aid measures:**Eye contact: rinse immediately with water, especially under eyelids, for >15 minutes. Consult a physician.  
Skin contact: wash off immediately with plenty of water and soap. Consult a physician.  
Inhalation: Move to fresh air. If not breathing, give artificial respiration. Consult a physician.  
Ingestion: Do not induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**Other hazards:**Fire: highly flammable liquid and vapor.   
Explosion: may form explosive mixtures with air.

**Cobalt (II) nitrate hexahydrate solution:**

CAS No.: 10026-22-9  
Molecular Weight: 291.03  
Chemical Formula: Co(NO3)2 · 6H2O  
Appearance: clear, pink liquid  
Lab Protective Equipment: Lab coat, goggles   
Oxidizer.

**Health effects:**Harmful if swallowed. May cause skin irritation, respiratory irritation.

**First Aid measures:**Eye contact: rinse immediately with water, especially under eyelids, for >15 minutes. Consult a physician.  
Skin contact: wash off immediately with plenty of water and soap. Consult a physician.  
Inhalation: Move to fresh air. If not breathing, give artificial respiration. Keep patient warm. Consult a physician.  
Ingestion: Do not induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**Other hazards:**Fire: reaction with reducing agents can cause fire.   
Explosion: not known to be an explosion hazard.

**Copper sulfate pentahydrate solution:**

CAS No.: 7758-99-8  
Molecular Weight: 249.69  
Chemical Formula: CuSO4 · 5H2O  
Appearance: clear, blue liquid  
Lab Protective Equipment: Lab coat, goggles

**Health effects:**Causes irritation and possible burns by all routes of exposure. Harmful if swallowed.

**First Aid measures:**Eye contact: rinse immediately with water, especially under eyelids, for >15 minutes. Consult a physician.  
Skin contact: wash off immediately with plenty of water, while removing contaminated clothing. Consult a physician.  
Inhalation: Move to fresh air. If not breathing, give artificial respiration, but not with mouth-to-mouth resuscitation. Consult a physician.  
Ingestion: Do not induce vomiting. Give 2-4 cupfuls of milk or water if conscious and alert. Never give anything by mouth to an unconscious person. Consult a physician.

**Other hazards:**Fire: not known to be an fire hazard.  
Explosion: not known to be an explosion hazard.

**Nickel (II) nitrate hexahydrate solution:**

CAS No.: 13478-00-7  
Molecular Weight: 290.8  
Chemical Formula: Ni(NO3)2.6H2O  
Appearance: clear, green liquid  
Lab Protective Equipment: Lab coat, goggles   
Strong oxidizer. Contact with other material may cause fire.

**Health effects:**May cause severe eye irritation and possible injury. Causes skin and respiratory tract irritation. May be harmful if swallowed.

**First Aid measures:**Eye contact: rinse immediately with water, especially under eyelids, for >15 minutes. Consult a physician.  
Skin contact: wash off immediately with plenty of water, while removing contaminated clothing. Consult a physician.  
Inhalation: Move to fresh air. If not breathing, give artificial respiration, but not with mouth-to-mouth resuscitation. Consult a physician.  
Ingestion: Do not induce vomiting. Give 2-4 cupfuls of milk or water if conscious and alert. Never give anything by mouth to an unconscious person. Consult a physician.

**Other hazards:**Fire: Contact with reducing agents may cause fire.  
Explosion: not known to be an explosion hazard.

**7) Post lab questions:**

1. Absorbance is measured in AU, or absorbance units. Molar absorptivity is measured in M-1cm-1, or L/(mol\*cm).
2. I would select the material based on the absorption wavelengths of the compounds I am measuring. If the compound absorbs in the visible spectrum, I would select glass or plastic, as it does not absorb in that spectrum. If it absorbs in the UV spectrum, I would select quartz, as that does not absorb in the UV spectrum.
4. UV-A, UV-B, and UV-C refer to different wavelengths within the UV region. UVC is the most energetic and damaging, with a wavelength between 100-280 nm. UVB is in the middle, with wavelength between 280-315 nm. UVC is the least energetic, with a wavelength between 315-400 nm.
5. A good sunscreen should absorb as much UV as possible. As UVC is completely absorbed by the atmosphere, and UVB is more damaging than UVA, as it has more energy, the ideal target would be to absorb as much UVB as possible. However, a mixture of chemical compounds could absorb both UVA and UVB wavelengths. These compounds should have chromophores, or conjugated pi bond systems, that are conjugated enough to target these wavelengths between 280-400 nm. As a single compound has a maximum absorption wavelength at only one wavelength, in the lab, we found that a mixture of compounds can cover a range of wavelengths. Thus, a good sunscreen might contain multiple compounds with conjugated pi systems of different sizes targeting wavelengths across the 280-400 nm region to absorb all the UV energy before it damages the skin.
6. Some chemicals are colored because of the chromophores in their chemical structures. When light hits a chemical, it can excite an electron to move from the HOMO (highest unoccupied molecular orbital) to the LUMO (lowest unoccupied molecular orbital). Because this requires a specific amount of energy, and different wavelengths of light have different energy, an electron will only jump when a specific wavelength of light hits it. Thus, this wavelength is absorbed by the chemical, and the chemical gains the color of light that results from reflecting all other colors. What wavelength is absorbed is based on chromophores, or conjugated pi-systems, and the size of the conjugated pi system. The larger the pi system, the longer wavelength the compound absorbs. Compounds that are colorless either do not have a chromophore or have a chromophore that does not absorb in the visible light spectrum, such as ascorbic acid, which absorbs in the UV region.
7. The chemical structure of quartz is a lattice structure of pure SiO2. Because this lattice structure means that each silicon atom is bonded to four oxygen atoms, there are no double bonds in quartz. Therefore, there are no chromophores, and quartz does not absorb any wavelengths, which would include UV light.
8. Frequency remains constant as light travels through different materials. Therefore: