UV-Visible Absorption Analysis of a Two Component System

1. Purpose

This procedure will determine the amounts of vanillin and \( p \)-hydroxybenzaldehyde in an unknown mixture using a UV-Vis spectrophotometer.

2. Background

Molecular absorption spectroscopy is based on the measurement of the transmittance (T) or the absorbance (A) of solutions contained in transparent cells having a path-length of \( b \) cm. Near-ultraviolet and visible light (160 – 780 nm wavelength) have sufficient energy to promote outer electrons to higher energy levels. When the wavelength of the incident radiation is equal to the difference in the discrete energy levels, the light is absorbed as the electron is promoted to an excited state. To determine transmittance, the power of radiation impinging on a sample cell (\( P \)) is compared to that of a reference cell containing no sample (\( P_0 \)), such that:

\[
T = \frac{P}{P_0}
\]

Absorbance is then calculated from transmittance:

\[
A = -\log T
\]

When absorbance is measured as a function of wavelength, an absorption band, also known as a spectrum, is produced. These absorption spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. When the intensity of the radiation is measured at a specific wavelength (\( \lambda \)), absorbance is related to the concentration of analyte as described by Beer’s Law:

\[
A = abc
\]

Where the proportionality constant \( a \) is the absorptivity (also known as extinction coefficient) of the species at the wavelength of interest, \( b \) is the pathlength of the sample (typically with units of cm), and \( c \) is the concentration. When \( c \) is expressed in grams per liter, absorptivity then has the units of liters per gram cm. When \( c \) is expressed as a molarity, the proportionality constant is \( \epsilon \), the molar absorptivity with units of liter per mol cm. This proportionality constant is specific to a species at a specific wavelength.

UV-vis spectroscopy is usually applied to molecules and inorganic ions or complexes in solution. It is a ubiquitous technique, with applications ranging from environmental science to the pharmaceutical industry. The light source is usually a deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible measurements.
instruments automatically swap lamps when scanning between the UV and visible regions. The wavelengths of these continuous light sources are typically dispersed by a grating in a single or double monochromator. The spectral bandpass is then determined by the monochromator slit width or by the array element width in array detector spectrometers. Spectrometer designs and optical components are optimized to reject stray light, which is one of the limiting factors in quantitative absorbance measurements. The detector in single-detector instruments is a photodiode, phototube, or photomultiplier tube (PMT).

In this particular analysis, the instrument used for analysis contains a diode array detector. Diode array detector spectrophotometers allow rapid recording of absorption spectra. Dispersing the source light after it passes through a sample allows the use of an array detector to simultaneously record the transmitted light power at multiple wavelengths.

In this laboratory, you will be given a sample of mixture of vanillin and $p$-hydroxybenzaldehyde. You will prepare the sample and standards for analysis in the spectrophotometer. Because absorbance is additive, you will be able to determine the weights of vanillin and $p$-hydroxybenzaldehyde in the original sample in grams. The structures of these molecules are shown below:

![Vanillin and $p$-Hydroxybenzaldehyde structures](image)

**Vanillin:**

4-Hydroxy-3-methoxybenzaldehyde  
$\text{max} = 347 \text{ nm}$

4-hydroxybenzaldehyde  
$\text{max} = 330 \text{ nm}$

3. **Materials and Equipment**

Vanillin  
$p$-hydroxybenzaldehyde  
potassium carbonate  
assorted glassware  
Agilent 8453 UV-Vis Diode Array Spectrophotometer  
Silica cuvette

4. **Safety**

Protective eyewear must be worn at all times. Notify the instructor if there is a problem with the instrument.
For more general safety in the laboratory, please refer the appendix.

5. Experimental Method

Preparation of Potassium Carbonate Solution

Prepare at least 0.5 L of 1 M potassium carbonate solution. Note, this solution is not a standard and so volumetric glassware is not required. The top-loader balance may be used along with a plastic bottle.

Preparation of the Stock Standard Solutions

Accurately weigh out between about 0.1 to 0.2 grams of vanillin to four decimal places on a weighing boat and record it in your notebook. Quantitatively transfer the vanillin with a wash bottle to a one liter volumetric flask. Add approximately 50 mL of potassium carbonate solution and swirl the flask until the solid completely dissolves. DO NOT CONTINUE TO THE NEXT STEP UNTIL YOU HAVE DISSOLVED ALL OF THE SOLID IN THE CARBONATE SOLUTION. THE ANALYTES MUST BE IN THIS BASIC ENVIRONMENT TO DISSOLVE.

Add nanopure water and bring the meniscus to the mark. Stopper and invert to mix. Transfer to a plastic bottle and label.

Accurately weigh out between 0.1 to 0.2 gram p-hydroxybenzaldehyde and follow the same solution preparation procedure as in step 5.2.1.

Preparation of Working Standards

Transfer a 10.00 mL aliquot of the standard vanillin to a one liter volumetric flask. Add approximately 50 mL of carbonate solution and dilute to the mark and mix. Transfer to a plastic bottle and label this "vanillin working standard". Calculate the concentration of this working standard, in g/L

Follow the procedure in step 5.3.1 for p-hydroxybenzaldehyde and label the new solution "p-hydroxybenzaldehyde working standard". Calculate the concentration of this working standard, in g/L

Note, it is not necessary to save the entire liter of either solution as only several hundred milliliters of each is needed.

Preparation of a Working Standard Mixture
Transfer a 10.00 mL aliquot of vanillin stock standard to a one liter volumetric flask. Transfer a 10.00 mL aliquot of p-hydroxybenzaldehyde stock standard to the same flask. Add approximately 50 mL of the carbonate solution. Dilute to the mark and transfer to a plastic bottle and label it "working standard mixture.” This solution will be used to test the additivity of Beer's Law.

**Preparation of the Stock Unknown Mixture**

Obtain an unknown from the instructor and record its number in your lab notebook. The components of this solid mixture have been weighed out by the instructor to four decimal places. Quantitatively transfer the unknown with a wash bottle to a one liter volumetric flask. Also rinse the container into the flask with some of the carbonate solution to ensure all of the sample has transferred. Add the carbonate to the flask (not to exceed 50 mL total carbonate solution). DO NOT CONTINUE TO THE NEXT STEP UNTIL YOU HAVE DISSOLVED ALL OF THE SOLID IN THE CARBONATE SOLUTION. THE ANALYTES MUST BE IN THIS BASIC ENVIRONMENT TO DISSOLVE. Dilute to the mark with nanopure water, and invert to mix. Transfer to a plastic bottle and label it "concentrated UV unknown".

**Preparation of Unknown Mixture for Analysis**

Quantitatively transfer a 5.00-mL aliquot of unknown stock solution to a one liter volumetric flask, add 50 mL of the carbonate solution, dilute to the mark with water, invert to mix and transfer to a plastic bottle. Label it "diluted unknown".

**Analysis Using the Agilent 8453 UV-Vis Diode Array Spectrophotometer**

Take the following to the instrument room:

1. Vanillin working standard
2. p-hydroxybenzaldehyde working standard
3. Known working standard mixture
4. Unknown diluted mixture
5. Wash bottle
6. Large empty beaker for rinsing

First, turn on the instrument, if necessary (Figure 1).
Start the software by double clicking the "HPUV-VIS" icon on the desktop (Figure 2).

![Lamp parameter window. You should turn on both Deuterium lamp and Tungsten lamp.](Figure 4)

Go to the "Instrument" menu and select "lamps". In the lamp parameter window, turn both the Deuterium lamp and Tungsten lamp on (Figure 4). You should hear a few clicks as the lamps turn on.

Switch to Spectrum/Peaks in the Task section (upper left of HP UV software — see Figure 5). At the Spectrum/Peaks Parameters window (Figure 6), deselect the "Find and annotate up to 3 valleys" box (the second check box from the top in the Peak/Valley find section), select "Prompt for sample information" box, and enter 300 and 400 in the two boxes in "Display spectrum" section.
Figure 5. Switch Spectrum/Peaks mode from Task section.

Thoroughly rinse the cuvettes several times with nanopure water. Run the blank (nanopure water): Ensure that the cuvette is clean and free of fingerprints or smudges. Cuvettes should be handled at the top on the frosted sides, or with gloves to prevent smudging from fingerprints. Insert the cuvette containing the blank solution into the sample cell holder. The light from the source runs from the back to the front in this instrument. Ensure that the clear side of the cuvette is facing the light. There is a lock on the left side of the cell holder (see Figure 7a-7c). Lock the cuvette in place by turning the lock lever vertically.

Once the blank is in place properly, click "Blank" button on the lower left corner, and wait. You may also push the “Blank” button on the instrument. A window will pop up displaying the blank scan once the instrument has taken the blank baseline. Hit “Blank” at least two additional times to ensure a stable blank.

Unlock the cell and remove the blank from the cell holder. Rinse the cuvette with the next solution to be analyzed (vanillin standard) several times. Fill the cuvette with the vanillin standard, place the cuvette in the cell holder, lock the cuvette, and click or push the "Sample" button. When prompted to enter the sample name, enter "vanillin" and click "OK".

Once the sample name has been entered, the absorbance values should be visible on the lower half of the UV-Vis windows (below the graph). Record the \( I_{max} \) for vanillin your notebook.

Repeat steps 5.7.9 and 5.7.10 with the \( p \)-hydroxybenzaldehyde standard solution.
At this point, print your result by clicking the printer icon at the top (see Figure 8).

Figure 8.
Use the print button at the tool bar to print.

Switch to the “Fixed Wavelengths” task at the top left corner (Figure 5 above). At the Fixed Wavelength(s) Parameters window, enter the \( \lambda_{\text{max}} \) values for vanillin and \( p \)-hydroxybenzaldehyde standard in the "Use wavelength(s)" boxes at the top and click "OK".

Rinse the cuvette and fill it with standard mixture solution. Place the cuvette in the sample cell holder and press "Sample" button.

Repeat step 5.7.14 for the unknown mixture.

Print the “Fixed Wavelengths” results (see Figure 8). The data should contain a total of eight absorbances values. Record these in your notebook.

Rinse the cuvette with nanopure water and place it the case.

Turn off the lamps from the lamp control window (see Figure 4).

Exit the software. As the software is exited, a window will pop up. Click "OK". Do not click "Save configuration" box. Do not turn off the computer.

Figure 10. Do not save the configuration.

6. Data Analysis/Calculations

Beer’s law will be used to determine the concentration of each species in the unknown mixture. Beer’s law is additive, so an expression similar to
\[ A_{total} = a_1bc_1 + a_2bc_2 \]

should be used. Remember that Beer’s law is for a specific wavelength.

First, use the individual standard data to determine the absorptivity of each individual species at each \( \text{max} \). Keep the concentration units in \( \text{g/L} \) and not molarity (M). Calculate all values to four significant figures.

Using the \( a \) values determined in step 6.2 and the absorbance of the working standard mixture, determine an expression to test the additivity of Beer’s law. Calculate the weight of vanillin and \( p \)-hydroxybenzaldehyde in the standard mixture and calculate the percent error for each component. This data will show how well the additivity holds. Typical errors are around 1-2\%, though less error obviously is preferable.

Using the \( a \) values determined in step 6.2 and the unknown mixture absorbances, calculate the mass of each species in the unknown. You will use two Beer’s law expressions, one for each wavelength from which the measurements were made. Use the expression in step 6.1.

7. **Reporting Requirements**

Calculate the weights of vanillin and \( p \)-hydroxybenzaldehyde in the original unknown sample in grams to four decimal places.

8. **Waste Disposal**

Discard all solutions in the sink.

9. **References**

Instrumental Analysis Laboratory Safety Rules

A. Instructions: Carry out all manipulations in accordance with instructions and the safety rules and procedures given herein.

B. Eye Protection: All students and staff working in the laboratory must wear safety glasses at all times.
   If a student needs to be reminded more than three times to wear goggles, she/he will be dismissed from lab for the remainder of the day, and will not be given an opportunity to make up the work.

C. Apparel: The clothes you wear in lab are an important part of your “safety equipment,” and should offer protection from splashes/spills. Closed toed shoes (sneakers are fine), Full-length pants or a full-length skirt, and A shirt that completely covers your torso (i.e. at minimum, a t-shirt).
    In other words, you must NOT wear shorts to lab. You must NOT wear flip-flops, sandals, or crocs. You must NOT wear tank tops, halter tops, spaghetti-strap tops, or low cut jeans to lab. Exposed abdomens, hips, and backs are not safe in the lab.

D. Gloves: Gloves are an important part of personal protection. Gloves will be available at all times in the laboratory. Your instructor will require their use when appropriate.

E. Food: Food, drinks, and gum are not allowed in lab. None at all, not even water bottles.

F. Sanitation Issues: Be sure to wash your hands before leaving lab, before you eat anything outside of lab, and before you answer your cell phone.

G. Music: Individual headphones are not allowed. Your may choose to play music for the entire class.

H. Other: All students are explicitly prohibited from:
   1. conducting any unauthorized experiments.
   2. removing chemicals or apparatus from the laboratory for any reason.
   3. working in the lab alone, or at other than regularly scheduled lab periods.
   4. smoking in the laboratory or within 20 feet of any doorway.
   5. impeding movement in aisles or through doorways with bags, skateboards, etc.