**Determination of Unknown Chemicals Using Absorption Spectroscopy**

Tara Walenczyk, Lindsey Riggs, Sebastian Ortiz

**Abstract:**

The absorbance of electromagnetic waves of a material can be used to determine unique characteristics of the material using the technique of absorption spectroscopy. Absorption spectroscopy is a method which uses the absorbance of a material across different electromagnetic wavelengths. The determination of absorbance across different wavelengths can be used to create an absorbance spectrum which is unique to the specific material. The specific material can then be determined by comparing the experimentally found absorption spectrum against known absorption spectrums.

**Introduction:**

Spectroscopy refers to the investigation of the emission, transmittance, and absorption of electromagnetic waves. Different sources of matter interact with electromagnetic radiation or waves in unique ways. Absorption spectroscopy encompasses the study of specifically how intensely materials absorb electromagnetic waves at different wavelengths. This technique can be used to determine characteristics of a solution such as the concentration of materials in a solution or the type of substance a solution is (“Absorption Spectroscopy”, 2020).

When light interacts with a medium, it will be refracted at an angle given by Snell’s Law, which depends on the refractive index (n) of the material, the refractive index of the incident material, and the incident angle of the light. The unique value of a material’s refractive index is why light is reflected differently when it comes in contact with different materials. The value of refractive indexes is dependent on the wavelength of the incident electromagnetic wave. Since different colors of light have different wavelengths, materials such as prisms and diffraction gradients diffract light of different wavelengths in different directions, separating wavelengths of different colors to create a rainbow.

Discovering the absorbance of a material at different wavelengths present in a rainbow can give information about the properties of a material. The intensity of absorbance of a material across different wavelengths can be found by first solving for the transmittance of light through the material across the varying wavelength spectrum, and then taking the negative log of that value. Normalizing the absorbance as a function of wavelength will give values necessary to create a graph of the material’s absorbance spectrum. Characteristics about the material, or in the case of our experiment, determination of what the material is, can be found by comparing the materials experimentally found absorbance spectrum to a known absorbance spectrum.

**Methods/Methodology:**

We set up a laser to shine towards a diffraction grading, creating a rainbow spectrum towards a detector connected to an oscilloscope. We set up the detector on a translating mount where we could precisely move the detector by a millimeter, 26 times, so that we could read the voltage across the colored spectrum created by the diffraction gradient. Every consecutive measurement, 0-26, that we recorded corresponded to about a 13.85 nm wavelength difference of light wave in the rainbow spectrum, because the wavelengths in the visible light spectrum are about 380nm-740nm and we took measurements over 26mm. We first proceeded to record the voltage read by the oscilloscope across the spectrum with no sample present by starting the detector at position 0mm or at a position where about 380nm of violet light were directed towards the detector. We moved the detector 1 mm to the right, recording the oscilloscope voltage measurement at each millimeter up to 26 millimeters.

Next, we placed our first unknown sample in a cuvette, and we taped the cuvette containing sample to the detector center to ensure the sample was directly over the detector and the tape was not covering the detector center. We then repeated the steps from above of moving the detector to obtain measurements of intensity of transmitted light in volts through the sample from 27 different wavelengths.

We calculated the transmittance of each sample at each wavelength we recorded data for by using the equation , where T is the transmittance through the sample as a function of wavelength, I(λ) is the intensity that the oscilloscope recorded going through the sample in volts, as a function of wavelength, and is the reference intensity in volts read by the oscilloscope without the sample in front of it at the same wavelength. Then, we calculated the absorbance of the sample from each data point by using the equation , where A is the absorbance of the sample as a function of wavelength and T is the transmittance through the sample as a function of wavelength. We then normalized this value by using the equation where A(λ)norm is the absorbance normalized, A(λ)minimum is the smallest A(λ) of the data set, and A(λ) maximum is the largest A(λ) of the data set. Then, we created an absorbance spectrum for sample one by plotting the calculated normalized absorbances versus wavelength. We repeated all of the steps completed to obtain the normalized absorbances versus wavelength for sample one, for sample two, and then again repeated every step for sample one and two for a second trial of both samples.

To determine the error of the laser at the end of our trials we recorded the voltage of the laser at the 26th millimeter every 30 second for ten minutes to find the variation in the laser voltage over time for an estimation of error in our experiment.

**Results:**

Our experimental set up is shown below, where the blue box is the light source, the back straight line is the light path, the black rectangle is the diffraction gradient, the rainbow is the diffracted light, the grey circle is the detector, the purple box is the oscilloscope, and the green rectangle is representing our sample.



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Sample calculation of the transmittance as a function of wavelength and then absorbance as a function of wavelength for trial 1 sample 1 at position 26 or at a wavelength of 740.1nm is shown below.

Calculation of error in voltage reading, by recording the change on oscilloscope voltage reading over ten minutes, every 30 seconds. The Maximum Value recorded over 10 minutes was 86.88mV, and the minimum value recorded over 10 minutes was 86.86mV, so there was a variation of ±.02mV.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Position | Wavelength (nm) | Voltage (mV)(reference) | Sample 1 T(λ) | Sample 1 A(λ) | Sample 1 A(λ) Normalized | Sample 2 Voltage | Sample 2 T(λ) | Sample 2 A(λ) | Sample 2 A(λ) Normalized |
| 26 | 740.1 | 80 | 0.775 | 0.225 | 0.389634 | 69.9 | 0.87375 | 0.12625 | 0.276915 |
| 25 | 726.25 | 78 | 0.75641 | 0.24359 | 0.421826 | 66.74 | 0.855641 | 0.144359 | 0.316635 |
| 24 | 712.4 | 76 | 0.671053 | 0.328947 | 0.56964 | 59.21 | 0.779079 | 0.220921 | 0.484566 |
| 23 | 698.55 | 76 | 0.513158 | 0.486842 | 0.843068 | 53.68 | 0.706316 | 0.293684 | 0.644164 |
| 22 | 684.7 | 75 | 0.466667 | 0.533333 | 0.923577 | 47.82 | 0.6376 | 0.3624 | 0.794884 |
| 21 | 670.85 | 73 | 0.438356 | 0.561644 | 0.972602 | 44.89 | 0.614932 | 0.385068 | 0.844605 |
| 20 | 657 | 72 | 0.430556 | 0.569444 | 0.986111 | 43 | 0.597222 | 0.402778 | 0.883448 |
| 19 | 643.15 | 71 | 0.422535 | 0.577465 | 1 | 39.84 | 0.561127 | 0.438873 | 0.96262 |
| 18 | 629.3 | 71 | 0.422535 | 0.577465 | 1 | 38.63 | 0.544085 | 0.455915 | 1 |
| 17 | 615.45 | 67 | 0.447761 | 0.552239 | 0.956316 | 38.28 | 0.571343 | 0.428657 | 0.940211 |
| 16 | 601.6 | 66 | 0.454545 | 0.545455 | 0.944567 | 38.59 | 0.584697 | 0.415303 | 0.910921 |
| 15 | 587.75 | 65 | 0.507692 | 0.492308 | 0.852533 | 39.23 | 0.603538 | 0.396462 | 0.869594 |
| 14 | 573.9 | 64 | 0.53125 | 0.46875 | 0.811738 | 38.64 | 0.60375 | 0.39625 | 0.86913 |
| 13 | 560.05 | 64 | 0.546875 | 0.453125 | 0.78468 | 39.02 | 0.609688 | 0.390313 | 0.856107 |
| 12 | 546.2 | 59 | 0.644068 | 0.355932 | 0.61637 | 39.26 | 0.665424 | 0.334576 | 0.733856 |
| 11 | 532.35 | 59 | 0.644068 | 0.355932 | 0.61637 | 39.86 | 0.675593 | 0.324407 | 0.71155 |
| 10 | 518.5 | 56 | 0.678571 | 0.321429 | 0.55662 | 40.08 | 0.715714 | 0.284286 | 0.623549 |
| 9 | 504.65 | 54 | 0.685185 | 0.314815 | 0.545167 | 39.88 | 0.738519 | 0.261481 | 0.573531 |
| 8 | 490.8 | 48 | 0.6875 | 0.3125 | 0.541158 | 39.83 | 0.829792 | 0.170208 | 0.373333 |
| 7 | 476.95 | 47 | 0.659574 | 0.340426 | 0.589517 | 38.62 | 0.821702 | 0.178298 | 0.391077 |
| 6 | 463.1 | 45 | 0.666667 | 0.333333 | 0.577236 | 38.01 | 0.844667 | 0.155333 | 0.340706 |
| 5 | 449.25 | 40 | 0.7 | 0.3 | 0.519512 | 35.48 | 0.887 | 0.113 | 0.247853 |
| 4 | 435.4 | 40 | 0.7 | 0.3 | 0.519512 | 34.25 | 0.85625 | 0.14375 | 0.3153 |
| 3 | 421.55 | 39 | 0.692308 | 0.307692 | 0.532833 | 33 | 0.846154 | 0.153846 | 0.337444 |
| 2 | 407.7 | 35 | 0.685714 | 0.314286 | 0.544251 | 32.43 | 0.926571 | 0.073429 | 0.161057 |
| 1 | 393.85 | 34 | 0.676471 | 0.323529 | 0.560258 | 29.26 | 0.860588 | 0.139412 | 0.305784 |
| 0 | 380 | 33 | 0.666667 | 0.333333 | 0.577236 | 29.21 | 0.885152 | 0.114848 | 0.251907 |

**Table 1.** First trial sample one and sample two volatage recorded, transmittence, absorbance and normalized absorbance.

**Graph 1.** Trial one normalized absorbance versus wavelength for sample one and two.

**Discussion:**

Our absorption spectrum from trial one sample one shows a trend of a lower absorbance at lower wavelengths and a higher absorbance between 550nm and 715nm, with a peak in absorbance at a wavelength of about 645nm of about .59. Our absorption spectrum for sample two in trial one shows a trend of a lower absorbance at lower wavelengths and a higher absorbance at wavelengths between 490nm and 740nm, with a peak in absorbance at about 635nm of about 1 which is higher than the peak of absorbance of sample one in the same trial. Our absorption spectrum for sample one in trial two does not show a general trend and shows many different peaks throughout the trendline. The absorption spectrum in trial two for sample two presents an overall trend of a lower absorbance at lower wavelengths and a higher absorbance between wavelengths of 495nm and 740nm, with a peak at about 629nm, which closely relates to values found in trial one for sample two. In trial one, sample one most closely relates to the absorption spectrum of chemical #2. In both trials one and two the absorption spectrum of sample 2 most closely relates to the absorption spectrum of chemical #1.

We calculated an error of about ±.02mV error in the voltage reading from the oscilloscope and therefore an error in the absorbance calculation in the absorbance spectrum which is represented by the standard error bars on the graphs. The lowest error was in the sample one calculation of trial one because it has the least variation in data, however all other error bars present an error of less than ±.2 normalized absorbance. Possible sources of error in this experiment could have been that the laser intensity changed more over time longer than 10 minutes, because it did take longer than 10 minutes to complete our trials. This could cause a larger error in the voltage shown by the oscilloscope at the higher wavelengths, or at position 26 which was recorded last. Also, the temperature or amount of light in the room on different days could have accounted for an error in the accuracy between our original reference voltages recorded and our second trial voltages recorded, which were measurements taken on different days. This could have led to the large error in the measurement of sample one in trial two.

**Conclusion:**

We discovered the normalized absorbance versus wavelength relationship of two unknown samples and were able to determine which chemical they were. The relationship for sample one in trial one most closely relates to the absorption spectrum of chemical #2, because it has an overall trend of lower absorbance at lower wavelengths and a higher absorbance between wavelengths of 550nm and 740nm. This trend also closely relates to chemical #1 but since sample 2 in our trials had a higher peak than sample 1 and the same overall trend, we determined that the sample 2 absorption spectrum more closely relates chemical #1.

We created the experimentally fount relationship between normalized absorbance and wavelength or absorption spectrum of out samples by using a diffraction gradient to separate different wavelengths of light and then reading the voltage recorded by a detector on an oscilloscope, calculating transmittance, absorbance, normalized absorbance, and graphing our calculated values.

**Bibliography:**

“Absorption Spectroscopy - What Is Absorption Spectroscopy.” *Ibsen Photonics*, 2020, ibsen.com/applications/spectroscopy/absorption-spectroscopy/.

**Extra Data:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Wavelength (nm) | Trial 2  Sample 1 Voltage | Trial 2 Sample 1 T(λ) | Trial 2 Sample 1 A(λ) | Trial 2 Sample 1 A(λ) Normalized | Trial 2 Sample 2 Voltage | Trial 2 Sample 2 T(λ) | Trial 2 Sample 2 A(λ) | Trial 2 Sample 2 A(λ) Normalized |
| 740.1 | 91.26 | 1.14075 | -0.05719 | 0.410864 | 56.33 | 0.704125 | 0.15235 | 0.168972 |
| 726.25 | 91.26 | 1.17 | -0.06819 | 0.212284 | 51.87 | 0.665 | 0.177178 | 0.246939 |
| 712.4 | 91.36 | 1.202105 | -0.07994 | -4.5E-05 | 46.31 | 0.609342 | 0.215139 | 0.366144 |
| 698.55 | 88.76 | 1.167895 | -0.0674 | 0.22641 | 42.58 | 0.560263 | 0.251608 | 0.480667 |
| 684.7 | 88.86 | 1.1848 | -0.07365 | 0.113689 | 37.36 | 0.498133 | 0.302654 | 0.640966 |
| 670.85 | 86.36 | 1.183014 | -0.07299 | 0.125523 | 34.28 | 0.469589 | 0.328282 | 0.721443 |
| 657 | 84.46 | 1.173056 | -0.06932 | 0.191826 | 31.16 | 0.432778 | 0.363735 | 0.832775 |
| 643.15 | 82.56 | 1.162817 | -0.06551 | 0.260586 | 28.83 | 0.406056 | 0.391414 | 0.919693 |
| 629.3 | 80.66 | 1.136056 | -0.0554 | 0.443203 | 27.52 | 0.387606 | 0.41161 | 0.983115 |
| 615.45 | 78.76 | 1.175522 | -0.07023 | 0.175349 | 25.65 | 0.382836 | 0.416987 | 1.000001 |
| 601.6 | 76.26 | 1.155455 | -0.06275 | 0.310405 | 25.67 | 0.388939 | 0.410118 | 0.97843 |
| 587.75 | 75.06 | 1.154769 | -0.0625 | 0.315059 | 27.52 | 0.423385 | 0.373265 | 0.862701 |
| 573.9 | 70.9 | 1.107813 | -0.04447 | 0.640667 | 28.61 | 0.447031 | 0.349662 | 0.788582 |
| 560.05 | 68.94 | 1.077188 | -0.03229 | 0.860551 | 29.56 | 0.461875 | 0.335476 | 0.744033 |
| 546.2 | 66.42 | 1.125763 | -0.05145 | 0.514595 | 30.69 | 0.520169 | 0.283855 | 0.581931 |
| 532.35 | 64.07 | 1.085932 | -0.0358 | 0.797134 | 31.89 | 0.540508 | 0.267197 | 0.529622 |
| 518.5 | 61.51 | 1.098393 | -0.04076 | 0.707645 | 33.16 | 0.592143 | 0.227574 | 0.405192 |
| 504.65 | 58.91 | 1.090926 | -0.0378 | 0.761148 | 34.99 | 0.647963 | 0.18845 | 0.282334 |
| 490.8 | 54.92 | 1.144167 | -0.05849 | 0.387407 | 34.98 | 0.72875 | 0.137421 | 0.122092 |
| 476.95 | 51.82 | 1.102553 | -0.0424 | 0.677993 | 35 | 0.744681 | 0.12803 | 0.092599 |
| 463.1 | 49.93 | 1.109556 | -0.04515 | 0.628336 | 33.13 | 0.736222 | 0.132991 | 0.108179 |
| 449.25 | 48.08 | 1.202 | -0.0799 | 0.000642 | 31.88 | 0.797 | 0.098542 | 0 |
| 435.4 | 44.41 | 1.11025 | -0.04542 | 0.623428 | 30.68 | 0.767 | 0.115205 | 0.052325 |
| 421.55 | 41.27 | 1.058205 | -0.02457 | 1.000002 | 29.4 | 0.753846 | 0.122717 | 0.075917 |
| 407.7 | 39.4 | 1.125714 | -0.05143 | 0.514933 | 27.54 | 0.786857 | 0.104104 | 0.017466 |
| 64.4 | 38.16 | 1.122353 | -0.05013 | 0.538388 | 25.08 | 0.737647 | 0.132151 | 0.105542 |
| 63.14 | 36.9 | 1.118182 | -0.04851 | 0.567592 | 24.06 | 0.729091 | 0.137218 | 0.121454 |

**Table 2.** Second trial sample one and sample two volatage recorded, transmittence, absorbance and normalized absorbance.

**Graph 2.** The normalized absorbance versus wavelength for the second trial of sample one and sample 2.