**Experiment 1: Spectroscopic Analysis of Two Analytes**

By: Irika Sinha

Partners: Jacob Stein, Karen Zhang

CHEM 145, Section AB

Introduction

In this experiment spectroscopy was used to eventually determine the concentration of two dyes in a solution of unknown composition (Unknown #1). Spectroscopy is particularly useful for finding the composition of an unknown since solutions of different colors absorb different wavelengths of light. The components of a mixture will absorb amounts of each wavelength that are proportional to the concentration of the component and the molar extinction coefficient of that component at the wavelength.

Beer’s Law for absorbance was particularly useful: it states that A = $εcl $where “A” is absorbance at a particular wavelength of light, “$ε$” is the molar extinction coefficient, “c” is the concentration of the solution, and “l” is the path length. It was used to determine the molar extinction coefficients of 30$ μ$M tartrazine and sunset yellow dye solutions.

The total absorbance of a mixture at a certain wavelength is the sum of the absorbance values of its components. $\frac{A\_{total}}{l}=\sum\_{1}^{n}ε\_{n}C\_{n}$ for each specific wavelength. These molar extinction coefficients were then used in a system of equations to determine the concentrations of tartrazine and sunset yellow dye in the unknown mixture.

Procedure/Observations

Procedure is as was written in the lab sheet. In brief, and with observations:

1. Prepare ~5mL of 30$ μ$M tartrazine and sunset yellow dyes
	1. For 30$ μ$M tartrazine: create 100mL of 780$μ$M in volumetric flask, dilute to 100mL 30$ μ$M tartrazine solution in new volumetric flask
	2. For 30$ μ$M sunset yellow: create 100mL 690$ μ$M in volumetric flask, dilute to 100mL of 30$ μ$M sunset yellow in new volumetric flask
2. Connect LabQuest
3. Measure absorbance spectra for tartrazine and sunset yellow, record peak absorbance wavelength and absorbance at that wavelength for both
4. Measure and record absorbance for tartrazine and sunset yellow at peak absorbance wavelengths for both
5. Repeat steps 3-4 for reference 30$ μ$M tartrazine and sunset yellow solutions
	1. Reference solutions were noticeably darker than the created 30$ μ$M solutions and had much higher absorbance values
	2. Peak absorbance wavelengths were very close
6. Record absorbance spectrum for unknown solution
	1. The peak absorbance wavelength was in between those of tartrazine and sunset yellow
7. Measure and record absorbance for unknown solution at peak absorbance wavelengths of reference 30$ μ$M tartrazine and sunset yellow solutions

Data

Part 1: Creating 30$ μ$M Solutions of Sunset Yellow and Tartrazine

|  |  |  |
| --- | --- | --- |
| Dye | Concentrated | 30uM Solution |
| Mass (mg) | Volume (mL) | Volume of Concentrated Solution (mL) | Total Volume (mL) |
| Sunset Yellow | 31 | 100 | 4.4 | 100 |
| Tartrazine | 42 | 100.91 | 3.9 | 100 |

Part 2: Absorbance Spectra, Peak Wavelengths, and Low Noise Measurements for Created Solutions of Sunset Yellow and Tartrazine

|  |  |  |
| --- | --- | --- |
| 30$ μ$M Dye Solution | Absorbance Spectrum | Low Noise Measurement |
| Peak Absorbance Wavelength (nm) | Absorbance | Average Absorbance at 479.9 nm | Average Absorbance at 414.8 nm |
| Sunset Yellow | 479.9 | 0.371 | 0.369 | 0.160 |
| Tartrazine | 414.8 | 0.558 | 0.152 | 0.543 |

Part 3: Absorbance Spectra and Peak Wavelengths for Reference Solutions Sunset Yellow and Tartrazine

|  |  |
| --- | --- |
| 30$ μ$M Dye Solution | Absorbance Spectrum |
| Peak Absorbance Wavelength (nm) | Absorbance |
| Sunset Yellow | 477.9 | 0.710 |
| Tartrazine | 415.7 | 0.930 |

Part 3: Low Noise Measurements for Unknown #1 and Reference Solutions Sunset Yellow and Tartrazine

|  |  |
| --- | --- |
| Solution | Low Noise Measurement |
| Average Absorbance at 477.9 nm | Average Absorbance at 415.7 nm |
| 30$ μ$M Sunset Yellow | 0.710 | 0.326 |
| 30$ μ$M Tartrazine | 0.279 | 0.943 |
| Unknown #1 | 0.437 | 0.730 |

Calculations

1. Concentrated Solution Creation
	1. Example: Sunset Yellow
	 $\left(\frac{31 mg ×\frac{1g}{1000mg}×\frac{1 mol Sunset Yellow}{452.37g Sunset Yellow}}{100.00 ml×\frac{1L}{1000ml}}×\frac{10^{6}μmol}{1 mol}\right)=690 μM$
2. Volume of Concentrated Solution to Create 30$ μ$M Solution
	1. Example: Sunset Yellow

$C\_{1}V\_{1}=C\_{2}V\_{2}$

$\left(\frac{31 mg ×\frac{1g}{1000mg}×\frac{1 mol Sunset Yellow}{452.37g Sunset Yellow}}{100.00 ml×\frac{1L}{1000ml}}×\frac{10^{6}μmol}{1 mol}\right)V\_{1}=\left(30 μM\right)\left(100.00ml\right) ∴$ $V\_{1}$= 4.4 mL

1. Maximum molar extinction coefficient $ε$ of provided reference solutions
	1. Example: Sunset Yellow at 477.9 nm

$A=εcl$

$\frac{A}{cl}=ε$

$\frac{0.710}{30μM×\frac{1mol}{10^{6}μmol}×1cm}=ε=2.4 × 10^{4}M^{-1}cm^{-1}$

1. Maximum molar extinction coefficient $ε$ of prepared solutions
	1. Example: Sunset Yellow at 479.9 nm

$A=εcl$

$\frac{A}{cl}=ε$

$\frac{0.710}{30μM×\frac{1mol}{10^{6}μmol}×1cm}=ε=1.2 × 10^{4}M^{-1}cm^{-1}$

1. Linear Unmixing for Unknown #1

Equation 1: $\frac{A\_{477.9nm}}{l}=ε\_{SY, 477.9nm}C\_{sunset yellow}+ε\_{T, 477.9nm}C\_{tartrazine}$

Equation 2: $\frac{A\_{415.7nm}}{l}=ε\_{SY, 415.7nm}C\_{sunset yellow}+ε\_{T, 415.7nm}C\_{tartrazine}$

$System: \left\{\begin{array}{c}0.437cm^{-1}=\left(2.4 × 10^{4}M^{-1}cm^{-1}\right)C\_{sunset yellow}+(9.3 × 10^{3}M^{-1}cm^{-1})C\_{tartrazine}\\0.730cm^{-1}=\left(1.1 × 10^{4}M^{-1}cm^{-1}\right)C\_{sunset yellow}+(3.1 × 10^{4}M^{-1}cm^{-1})C\_{tartrazine}\end{array}\right.$

Solve the system of equations:

Solve equation 1 for Csunset yellow: $C\_{sunset yellow= }$($1.8×10^{-5})-0.39C\_{tartrazine}$

Substitute the equation for sunset yellow into the second equation:

$$0.730cm^{-1}=\left(1.1 × 10^{4}M^{-1}cm^{-1}\right)((1.8×10^{-5})-0.39C\_{tartrazine})+(3.1 × 10^{4}M^{-1}cm^{-1})C\_{tartrazine}$$

Solve for Ctartrazine: $C\_{tartrazine}=2.0 × 10^{-5}M$

Substitute Ctartrazine value into equation 1 and solve: $C\_{sunset yellow}=1.1 × 10^{-5}M$

Results

Part 1 Concentrations for Prepared Solutions

|  |  |  |
| --- | --- | --- |
| Dye | Concentrated Solution Molarity | Volume Concentrated Solution Diluted to Create 30 $μ$M Solution |
| Sunset Yellow | 690 $μ$M | 4.4mL |
| Tartrazine | 780$ μ$M | 3.9mL |

Maximum Molar Extinction Coefficients, $ε$, of Solutions

|  |  |  |
| --- | --- | --- |
| Dye | Prepared Solutions Molar Extinction Coefficients ($M^{-1}cm^{-1})$ | Reference Solutions Molar Extinction Coefficients ($M^{-1}cm^{-1})$ |
| 479.9 | 414.8 | 477.9 | 415.7 |
| Sunset Yellow | $$1.2 × 10^{4}$$ | $$5.3 × 10^{3}$$ | $$2.4 × 10^{4}$$ | $$1.1 × 10^{4}$$ |
| Tartrazine | $$5.1 × 10^{3}$$ | $$1.8 × 10^{4}$$ | $$9.3 × 10^{3}$$ | $$3.1 × 10^{4}$$ |

Dye Concentrations in Unknown #1

|  |  |
| --- | --- |
| Solution | Concentration of Dye (M) |
| Sunset Yellow | Tartrazine |
| Unknown #1 | $$1.1 × 10^{-5}$$ | $$2.0 × 10^{-5}$$ |

Discussion

 Calculations I and II are concerned with preparing 30 uM solutions of sunset yellow and tartrazine dyes from scratch. This was done by first creating concentrated solutions of the dyes, 690 $μ$M for sunset yellow and 780$ μ$M for tartrazine, and then diluting those solutions to 100 $mL$ of 30$ μ$M solutions of each. The average absorbance values of each were taken and then used to calculate molar extinction coefficients. As seen in the graphs, the absorbance was fairly constant at each wavelength, for each dye, so the average is a valid measurement.

The molar extinction coefficients for each dye at each wavelength is a constant, no matter the concentration of the dye in solution. By finding the molar extinction coefficients for both sunset yellow and tartrazine at the peak absorbance wavelengths for both, a total of four molar extinction coefficients were identified for each set of dye solutions. Unfortunately, the absorbance values and so the molar extinction coefficients for the dyes were much smaller for the prepared solutions than the reference solutions provided. At their peak wavelengths in the prepared solutions, sunset yellow had an extinction coefficient of $1.2 × 10^{4}M^{-1}cm^{-1}$ and tartrazine had one of $1.8 × 10^{4} M^{-1}cm^{-1}$. In the reference solutions these extinction coefficients were $2.4 × 10^{4} M^{-1}cm^{-1}$ and $3.1 × 10^{4}M^{-1}cm^{-1}$ respectively. This discrepancy likely occurred due to negligence in reading the dye bottle labels. The masses used to create the concentrated solutions assumed that the dye in the bottle was pure, but apparently it was not 100% dye. As a result, the concentrations of the prepared dyes were lower than 30 $μ$M and the solutions did not absorb as much light. During the experiment the spectrophotometer was not covered, so it is also possible that sunlight entered the spectrophotometer during the prepared solutions’ measurements and adversely affected them.

The extinction coefficients measured for the reference solutions were then used in a system of equations to identify the concentrations of sunset yellow and tartrazine dye in the Unknown #1 solution. The extinction coefficients at the peak absorbance wavelengths were used because they would be most distinctive for each type of dye. Other wavelengths could have been used, although wavelengths at which the absorbance was the same for tartrazine and sunset yellow would have caused ambiguity. These concentrations were found to be $1.1 × 10^{-5}M$ and $2.0 × 10^{-5}M$ respectively. The peak on the absorbance spectrum graph for Unknown #1 is closer to the peak for tartrazine than that of sunset yellow’s, so it makes sense that there is a higher concentration of tartrazine than sunset yellow in the unknown solution. Although “linear unmixing” was used for only two analytes in this experiment, it could be used for an unknown with three or even more analytes. The solution would simply have to be analyzed at more wavelengths and since there would be more unknowns in the system of equations.

Questions

1. The maximum molar coefficients of the reference solution were $2.4 × 10^{4} M^{-1}cm^{-1}$ and $9.3 × 10^{3} M^{-1}cm^{-1}$ at 477.9 nm and $1.1 × 10^{4} M^{-1}cm^{-1}$ and $3.1 × 10^{4} M^{-1}cm^{-1}$ at 415.7 nm for 30$ μ$M sunset yellow and tartrazine respectively.
2. See step 1 of the procedure for creation of 30$ μ$M solutions of tartrazine and sunset yellow dyes.
3. The maximum molar coefficients of the prepared solution were $1.2 × 10^{4} M^{-1}cm^{-1}$ and $5.1 × 10^{3} M^{-1}cm^{-1}$ at 479.9 nm and $5.3 × 10^{3} M^{-1}cm^{-1}$ and $1.8 × 10^{4} M^{-1}cm^{-1}$ at 414.8 nm for 30$ μ$M sunset yellow and tartrazine respectively. These extinction coefficients are much lower than the ones for the TA-provided solutions. This is likely due to not noticing that the bottles of dye were not 100% dye and so creating solutions of much lower concentration than expected.
4. See “Results” for the graph of absorbance vs. wavelength.
5. Using linear unmixing, in Unknown #1 the concentration of tartrazine is $2.0 × 10^{-5} $M and the concentration of sunset yellow is $1.1 × 10^{-5} M.$
6. Benefits of choosing the peak absorbance wavelength include distinctive molar extinction coefficients for the dyes at a certain wavelength. It would only be disadvantageous to use the peak absorbance wavelengths if they are similar for the components of the solution. However, it is not necessary to use the peak absorbance wavelengths. Other sets of wavelengths would also work fine, as long as the molar extinction coefficients are different from one another. Wavelengths in which the components have the same absorbance would not be useful.
7. Linear unmixing can be used for as many unknown components as desired. The solution would simply have to be analyzed at as many wavelengths as components since the system of equations would include more unknown concentrations.
8. The purpose of the “blank” measurement when setting up the spectrophotometer is to set a baseline for absorbance. The amount of light that passes through the cuvette and can be measured by the spectrophotometer is set as “0” for a blank
9. The equipment might be limited to an absorbance of 1.5 because as the absorbance gets higher, the amount of incoming light transmitted to the detector gets very small. At absorbance values higher than 1.5, it is likely that the amount of light transmitted to the detector is too small to measure. At an absorbance of 1.5, about $\frac{1}{32}$ of the incoming light is transmitted to the detector. If the path length was doubled, the absorbance would double and $\frac{1}{1000}$ of the incoming light would be transmitted to the detector. As the absorbance increases, the amount of light transmitted to the detector because tiny.
10. The relationship between the wavelength of light that the solution absorbs and the visible color is that when light passes the solution, the light not absorbed will be reflected and will be the complementary color of the wavelength(s) absorbed.



Darker blue

red

green

yellow

orange

blue

purple

As seen in this diagram: absorbance of 477.9nm will make a substance orange, as sunset yellow was, and absorbance of 415.7nm will make a substance, such as tartrazine, yellow.

Works Cited

Reusch, William. “Visible and Ultraviolet Spectroscopy.” *Spectroscopy*, Michigan State University, 5 May 2013, www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/uv-vis/spectrum.htm.