# JoVE: Science Education

## Method of Standard Additions (MOSA)

--Manuscript Draft--

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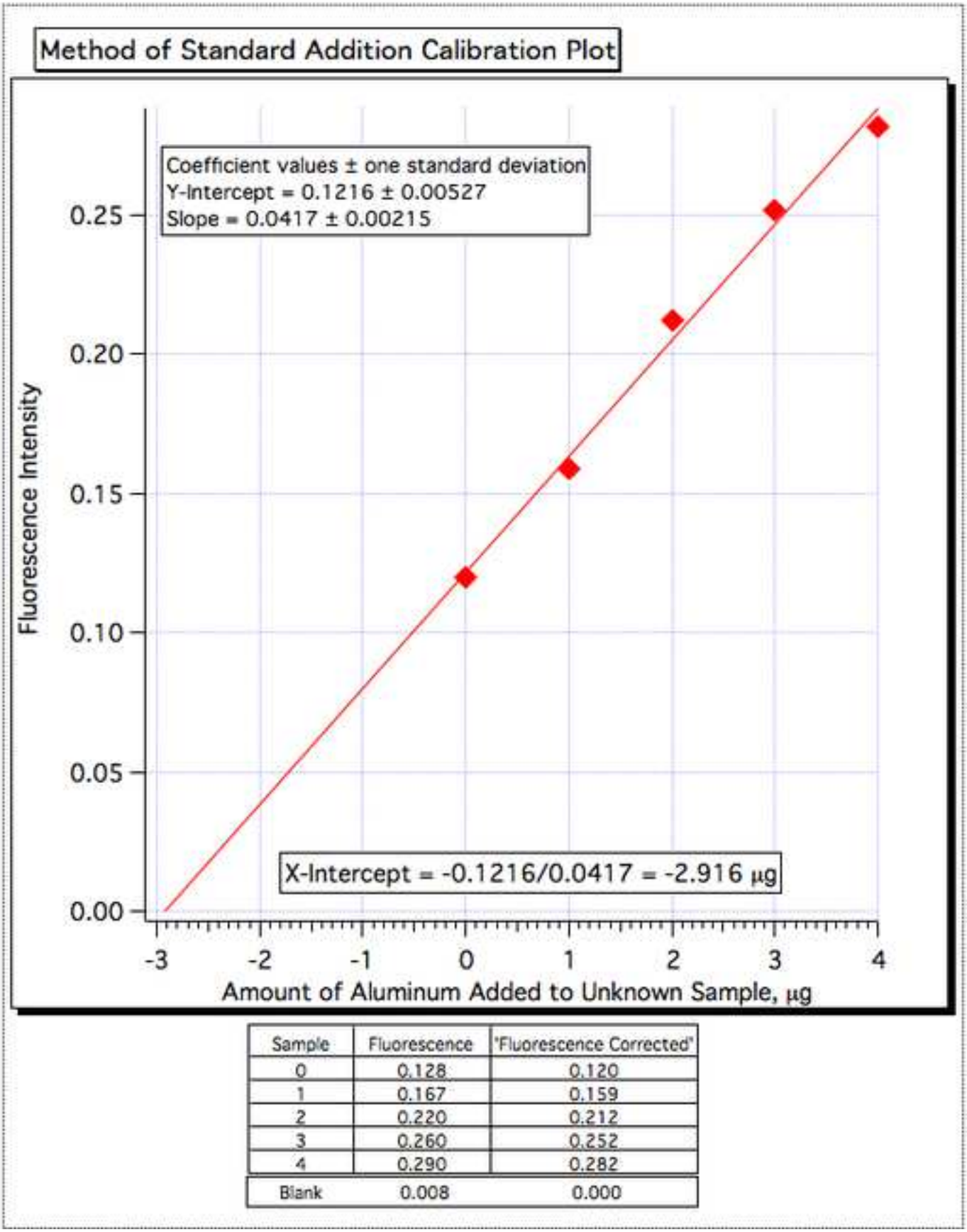


| Sample | Fluorescence | "Fluorescence Corrected" |
|---|---|---|
| 0 | 0.128 | 0.120 |
| 1 | 0.167 | 0.159 |
| 2 | 0.220 | 0.212 |
| 3 | 0.260 | 0.252 |
| 4 | 0.290 | 0.282 |
| Blank | 0.008 | 0.000 |

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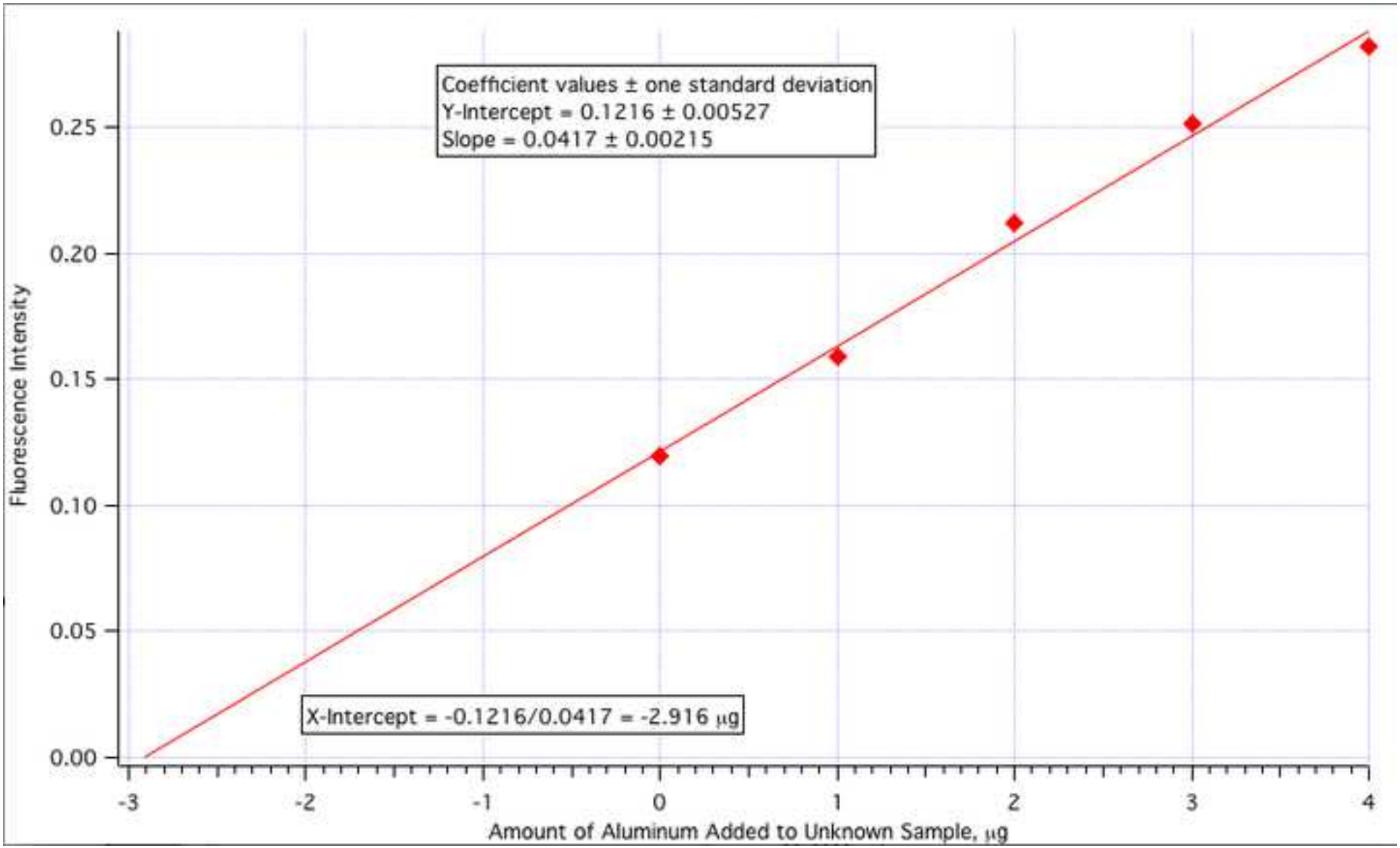

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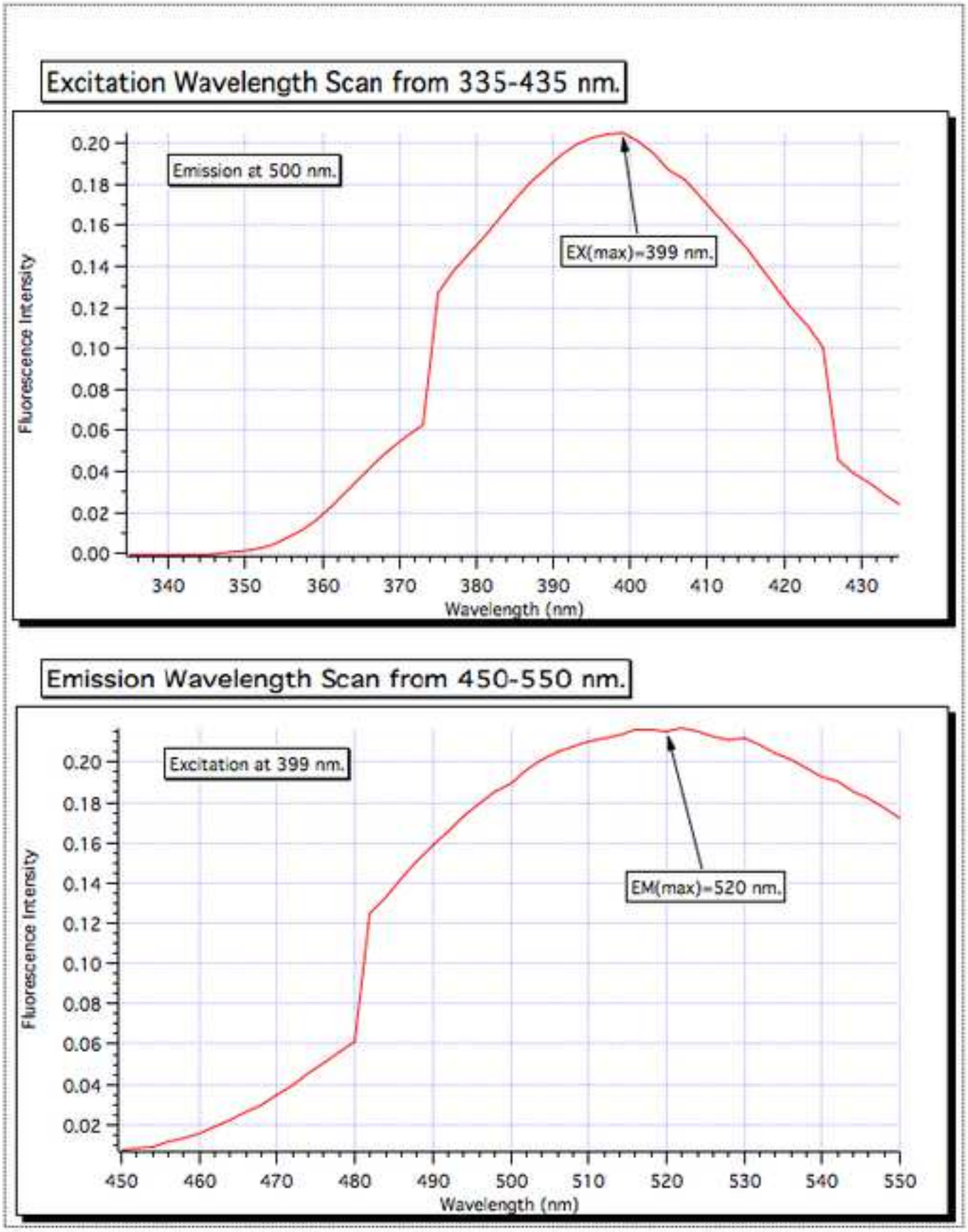

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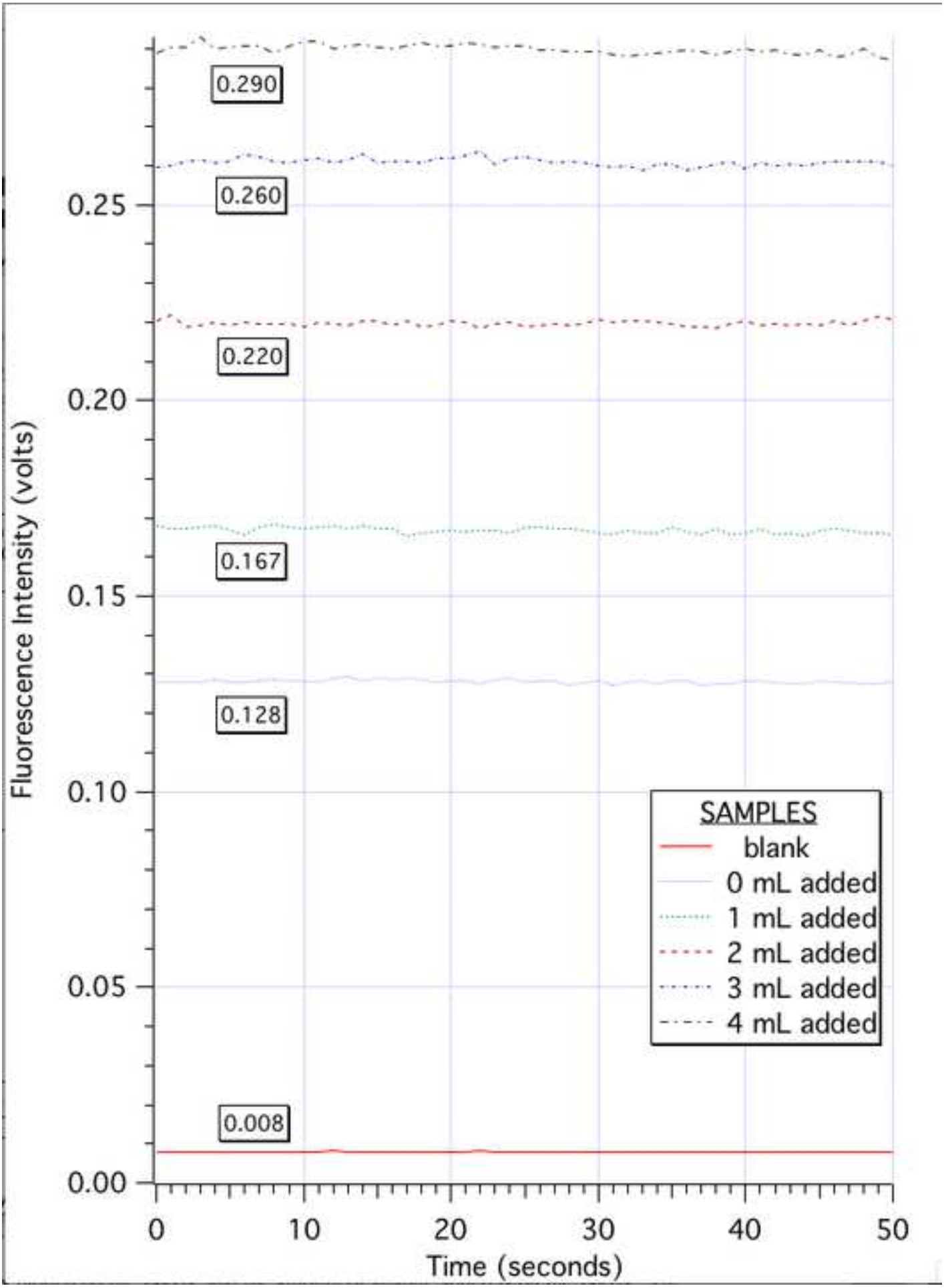

***PI: Paul Bower, Purdue***

***Science Education Chemistry Experiment: Method of Standard Additions (MOSA)***

## Overview:

The Method of Standard Additions is a quantitative analysis method which is often used when the sample of interest has multiple components that result in a matrix effect, where the additional components may either reduce or enhance the analyte absorbance signal. That results in significant errors in the analysis results.

MOSA is commonly used to eliminate matrix effects from a measurement, since it is assumed that the matrix affects all of the solutions equally. Additionally, it is used to correct for the chemical phase separations performed in the extraction process.

MOSA is performed by reading the experimental (in this case fluorescent) intensity of the unknown solution and then by measuring the intensity of the unknown with varying amounts of known standard added. The data are plotted as fluorescence intensity vs. ~~concentration~~ the amount of the standard **added** (the unknown itself, with no standard added, is plotted ON the y-axis). The Least Squares Line intersects the x-axis at the negative of the concentration of the unknown, as shown in **Figure 1**.

~~This quantitative analysis method is often used when the sample has multiple components that result in a matrix effect, where the additional components may either reduce or enhance the analyte absorbance signal. That results in significant errors in the analysis results.~~

## Principles:

In this experiment, the Method of Standard Additions (MOSA) is demonstrated as an analytical tool. MOSA is a procedure for the quantitative analysis of a species without the generation of a typical calibration curve. MOSA analysis is accomplished by measuring spectroscopic intensity before and after the addition of precise aliquots of a known standard solution of the analyte.

~~One way to overcome the selectivity limitation is to study~~This experiment studies non-fluorescent species by reacting them in such a way as to form a fluorescent complex. This approach ~~, which will be exploited in this lab,~~ is commonly used in the investigation of metal ions. Aluminum ions ($Al^{3+}$) will be determined by forming a complex with 8-hydroxyquinoline (8HQ). The $Al^{3+}$ is precipitated by 8HQ from aqueous solution and then is extracted into chloroform; the fluorescence of the chloroform solution is measured and related to the concentration of the original $Al^{3+}$ solution. Sensitivity in the part-per-million (ppm *or* µg/mL) range is expected for this experiment.

The reaction is

$$Al^{3+} + \text{(8HQ)} \xrightarrow[pH\sim 8]{NH_4^+/NH_3} Al(8HQ)_3$$

*(yellow ppt)*

The ppt is soluble in $CHCl_3$.

~~In this experiment, the Method of Standard Additions (MOSA) is demonstrated as an analytical~~



**Commented [ASW1]:** The first 2 sections should focus on the why and how of standard additions, and less on the specific reaction that will be studied (though it can still be discussed). Focus on when this technique is used (including some examples of common matrix effects); how it compares to calibration curves, etc.

**Commented [ASW2]:** Begin the Overview with this concept: what SA is, and what it is used for.

**Commented [ASW3]:** Explain how MOSA is utilized here.

~~tool. MOSA is a procedure for the quantitative analysis of a species without the generation of a calibration curve. MOSA analysis is accomplished by measuring spectroscopic intensity before and after the addition of precise aliquots of a known standard solution of the analyte. MOSA is commonly used to eliminate matrix effects from a measurement, since it is assumed that the matrix affects all of the solutions equally. Additionally, MOSA is used to correct for the chemical phase separations performed in the extraction process.~~

| | |
|---|---|
| Blank | 0 |
| Unk + 0 mL STD | $V_{UNK}(C_{UNK}) = 25\ mL(C_{UNK})$ |
| Unk + 1 mL STD | $V_{UNK}(C_{UNK}) + V_{STD}(C_{STD}) = 25\ mL(C_{UNK}) + 1\ mL(1\ \mu g/mL)$ |
| Unk + 2 mL STD | $V_{UNK}(C_{UNK}) + V_{STD}(C_{STD}) = 25\ mL(C_{UNK}) + 2\ mL(1\ \mu g/mL)$ |
| Unk + 3 mL STD | $V_{UNK}(C_{UNK}) + V_{STD}(C_{STD}) = 25\ mL(C_{UNK}) + 3\ mL(1\ \mu g/mL)$ |
| Unk + 4 mL STD | $V_{UNK}(C_{UNK}) + V_{STD}(C_{STD}) = 25\ mL(C_{UNK}) + 4\ mL(1\ \mu g/mL)$ |

**Commented [ASW4]:** Begin the Overview with this concept: what SA is, and what it is used for.

**Commented [ASW5]:** Explain how MOSA is utilized here.

## Procedure:

## 1. Preparing the Reagents

1.1 100 ppm standard $Al^{3+}$ solution: Dissolve 0.9151 g~~-~~ a~~A~~luminum n~~N~~itrate ($Al(NO_3)_3$•~~-~~$9H_2O$) into a 1.0 L volumetric flask with DI water.

1.2 8HQ solution in 1 M acetic acid (2% wt/vol): Add 2.0 g~~-~~ of 8-h~~H~~ydroxyquinoline to a 100 mL volumetric flask.

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1.3 Carefully add 5.74 mL glacial acetic acid to the 100 mL flask, then dilute to the mark with DI water. This ~~will~~ allows the 8-hydroxyquinoline to dissolve in aqueous phase.

**Commented [ASW6]:** Is this to lower the pH?

1.4 1 M $NH_4^+/NH_3$ buffer (pH~8): Add 20 g~~-~~ of ammonium acetate ($NH_4OAc$) to a 100 mL bottle.

**Commented [ASW7]:** Is there a specific reason this solution needs to be buffered?

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1.5 Add 7 mL of 30% ammonium hydroxide to this 100 mL bottle, and dilute to the mark with DI water. This ~~will~~ helps neutralize the acid in the 8HQ solution when combined.

1.6 Other reagents include anhydrous sodium sulfate ($Na_2SO_4$) and chloroform (Spec grade).

## 2. Preparing the Samples

2.1 Prepare a 1.00 ppm standard $Al^{3+}$ solution by adding 1.0 mL of the 100 ppm stock $Al^{3+}$ solution with a pipet to a 100 mL volumetric flask.

2.2 Place six 125~~-~~mL separatory funnels onto the rings that are on a large ring stand located in the hood. They should be labeled as follows: BL, 0, 1, 2, 3, 4. Make sure all glassware is scrupulously clean, as it is difficult to obtain quantitative results if little beads of chloroform stick to the walls of the glassware.

2.3 Add 25.00 mL of the unknown $Al^{3+}$ solution to the five separatory funnels labeled 0, 1, 2, 3 & 4. In this example, the unknown concentration is 0.110 ppm.

2.4 Add 0, 1.00, 2.00, 3.00 and 4.00 mL of the 1.00 ppm standard $Al^{3+}$ solution, respectively, to the five funnels with a 1.0 mL pipet.

2.5 Prepare a BLANK by adding 25.00 mL of distilled water to the separatory funnel labeled BL.

2.6 Add 1.0 mL of the 8-hHydroxyquinoline solution with a pipet to each of the six solutions.

2.7 Add 3.0 mL of buffer solution with a pipet to each of the six solutions.

2.8 Extract each solution twice with 10 mL of chloroform, shaking vigorously for 1 min each time. *Remember to occasionally vent the separatory funnel to release pressure buildup.* (NOTE: A good extraction only takes place when there is lots of liquid-liquid contact between the phases).

2.9 Collect the chloroform in a clean, dry 100 mL labeled beaker. Chloroform has a density of close to 1.5 $g/cm^3$, so it is the lower layer. There should be no trace of yellow color left in the aqueous phase after a complete extraction.

2.10 Transfer the combined chloroform extract from each beaker into their respective 25 mL volumetric flask and dilute to the mark with chloroform. Be sure to place to stoppers in each volumetric flask to keep any chloroform from evaporating.

2.11 Add ~1 g of anhydrous sodium sulfate ($Na_2SO_4$) to each of the six 100 mL beakers from step 2.9. The sodium sulfate ~~will~~ helps remove any trace of water that may be present in the chloroform extract.

2.12 Transfer the solutions back to their respected beakers. Swirl carefully to facilitate dehydration of any water in the sample.

2.13 Decant the chloroform extracts into a quartz fluorimeter cell (*chloroform will dissolve a plastic polystyrene cell*).

## 3. Selecting the Excitation Wavelength

Determine the excitation and emission wavelengths by running scans, then simply read and record the fluorescence intensity of all samples at those values. The excitation and emission bandwidths are preset at 5 nm. The complex absorbs in the near UV, so the excitation wavelength should be about 385 nm. Initially, monitor the fluorescence at 500 nm in the emission branch.

3.1 ~~Make sure~~On the Fluorimeter, verify that both the internal and outer cooling fans in the are turned on prior to powering up the xenon lamp. The Xenon lamp gets very hot, and requires continual cooling.

**Commented [ASW8]:** Why is this step done?

**Commented [ASW9]:** Sections 3 and 4 are getting away from the concept of "standard additions". Condense down to not detract from the main point.

3.2

3.~~5~~3 Open both of the shutters.

3.4 Open the data acquisition program "100nmFluorScan" on the computer.

3.5 Place the "Sample + 2 mL added" (2) solution into the quartz cell to use for determining the best excitation and emission wavelengths.

3.~~6~~6 ~~Send the fluorimeter excitation to the starting wavelength of 335 nm. EX, LOWER, 335, ENTER~~

3.~~13~~7 From the resulting fluorescence plot, determine the maximum fluorescence wavelength for the excitation, and set the instrument to that EX λ value.

## 4. Selecting the Emission Wavelength

4.1 Set the fluorimeter emission wavelength to 450 nm.

4.2 Set the emission wavelength range to run a 100 nm scan from 450-550 nm.

4.3 To start the scan, click the Start Trial button on the program at the same time of pressing the START button on the front panel of the fluorimeter.

4.4 From the resulting fluorescence plot, determine the maximum fluorescence wavelength for the emission, and set the instrument to that EM λ value (**Figure 2**).

## 5. Measuring the Fluorescence of the Samples

5.1 All samples are run at $EM\lambda_{max}$ and $EX\lambda_{max}$. Scans are not needed for each sample, but just the fluorescence value at these conditions. Starting with the most dilute sample (blank), place in a quartz cell and then in instrument. Record the fluorescent intensity in lab notebook.

5.2 Repeat for all other samples.

5.3 Remember that the Blank Relative Intensity must be subtracted from the Relative Intensities of each solution prior to creating the calibration chart.

## 6. Creating the ~~Calibration Chart~~Standard Addition Plot

6.1 Plot the fluorescence intensity vs. μg of $Al^{3+}$ added.

**Commented [ASW10]:** This is a bit too specific. We want to keep procedures more general.

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**Commented [ASW12]:** Is this the proper term? Not "Standard Addition Plot"?

6.2 Determine the least-squares value of the resulting plot, and record both the slope and intercept.

6.3 Determine the μg of $Al^{3+}$ in the unknown sample from the equation μg of $Al^{3+}$ = -b/m

6.4 Knowing that the unknown aluminum had a volume of 25.0 mL added to each sample, determine the concentration of the aluminum in the unknown.

## Representative Results:

A scan of the excitation wavelength from 335-435 showed the highest absorption at 399 nm, so the excitation monochromator was set for that value. Then the emission scan was performed from 450-550 nm, and the strongest signal was found to be at 520 nm. These are the wavelengths that are used for all of the samples.

| SAMPLE | Fluorescence Intensity | Corrected Fluorescence Intensity |
|---|---|---|
| Blank | 0.008 | 0.000 |
| Sample | 0.128 | 0.120 |
| Sample + 1 mL | 0.167 | 0.159 |
| Sample + 2 mL | 0.220 | 0.212 |
| Sample + 3 mL | 0.260 | 0.252 |
| Sample + 4 mL | 0.290 | 0.282 |

A plot of fluorescence (**Figure 3**) vs. μg of $Al^{3+}$ added (**Figure 4**) yielded a least-squares line of:

Fluorescence Intensity = 0.0417 x (μg of $Al^{3+}$ added) + 0.1216

Amount of $Al^{3+}$ = -(Y-Int)/Slope = -0.1216/0.0417 = -2.916 μg/mL

Since the amount of unknown added was 25 mL, then the 2.916 μg/mL value needs to be divided by 25.

Unknown Aluminum Concentration = 2.916 μg/mL / 25.0 mL = 0.117 μg/mL = 0.117 ppm which is quite close to the actual value of 0.110 ppm (6.4% error).

## Applications:

The method of standard additions is often the technique utilized when accurate quantitative results are desired, used in analytical analysis such as atomic absorption, fluorescence spectroscopy, ICP-OES, and gas chromatography. This is often used when there are other components in the sample of interest that causes either a reduction or enhancement of the absorbance desired for quantitative results. When this is the case, one cannot simply compare the analytes signal to standards using the traditional calibration curve approach. In fact, matrix effect evaluation should be a mandatory part of the validation procedure.

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**Commented [ASW13]:** What demonstration and/or media can you provide for these Applications.

**Commented [DM14R13]:** The author struggled here Andrew. He seems to indicate that he doesn't have any. Aside from what he wrote here, he said he found another application online where MOSA is used to determine the Mn content in steel, but all he could provide for that was a URL, which he didn't think was what we were after.

Hopefully the other modifications he made will suffice and we can figure out how to tackle this section as-is. Let me know how/if I can help.

Another example where MOSA can be used is when extracting silver from old photographic waste. The waste contains silver halides, and can be extracted so the silver can be reclaimed. By spiking the unknown "waste" with known amounts of silver, this method can predict the amount of silver obtained from the photographic film.

Workers who are exposed to benzene manufacturing plants are often tested to verify they are safely below the accepted levels of benzene. Their urine is tested for the chemical, and that is the biological matrix. Also, the amount of analyte suppression varies for different people, so a single calibration kit will not work. With the method of standard addition, every employee can be tested and evaluated accurately.

**Legend:**
**Figure 1:** Graphic representation of method of standard addition (MOSA)
**Figure 2:** Determining optimum EX and EM Wavelengths
**Figure 3**: Fluorescence of the Samples
**Figure 4:** The MOSA Calibration Plot