

## **Lab 2: Quantitative Dilution and Solution Preparation**

### **Questions**

1. I expect the concentration of the final solution to be  $40 \text{ mg/mL} \times \frac{0.05 \text{ mL}}{10 \text{ mL}} \times \frac{0.25 \text{ mL}}{25 \text{ mL}} = 0.002 \text{ mg/mL} = 0.002 \text{ mg/mL} \times 10^6 \text{ ng/mL} = 2 \times 10^3 \text{ ng/mL}$ . The absorbance can be calculated as follows (copied from prelab)

$$A_\lambda = c\epsilon_\lambda l$$

$$A_{503 \text{ nm}} = (2.00 \times 10^3 \text{ ng/mL})(2.59 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1})(1.0 \text{ cm})$$

$$A_{503 \text{ nm}} = (2.00 \times 10^3 \text{ ng/mL})(10^{-9} \text{ g/ng})(10^3 \text{ mL/L})(2.59 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1})(1.0 \text{ cm})$$

$$A_{503 \text{ nm}} = (2.00 \times 10^{-3} \text{ g/L})(2.59 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1})(1.0 \text{ cm})$$

$$A_{503 \text{ nm}} = (20.0 \text{ g})(2.59 \text{ mol}^{-1})(1.0)$$

$$A_{503 \text{ nm}} = (20.0 \text{ g})(2.59 \text{ mol}^{-1})(1.0) \frac{1 \text{ mol}}{496.42 \text{ g}}$$

$$A_{503 \text{ nm}} = 0.10$$

CHEM2322 Experiment 2 Procedure

Author: Lukas Rosipko

**Procedure:**

1. First, acquire the 0.1 mL of 40.0 mg/mL red 40 stock solution, a 10 mL volumetric flask, a 25 mL volumetric flask, and 2 stoppers for the volumetric flasks.
2. Rinse both volumetric flasks with deionized water three times. Use a funnel as needed.
3. The first dilution will use the 10 mL volumetric flask. Transfer ~~0.05~~ <sup>1.0</sup> mL of the 40.0 mg/mL red 40 stock solution into the 10 mL volumetric flask using a micropipette.
4. Fill a beaker with deionized water and subsequently use a transfer pipette to carefully fill the 10 mL volumetric flask to the mark in the neck, ensuring the meniscus falls on the mark.
5. Place a stopper in the 10 mL volumetric flask and twist to ensure a good seal. Place your thumb over the top of the stopper and invert the flask, so the air bubble rises to the top (bottom of the flask) and then turn the flask right side up. Invert the 10 mL volumetric flask with this process 20 times.
6. Remove the stopper and do not place it on the lab bench, continuing to hold it during the remainder of this step. Transfer 0.25 mL of the solution present in the 10 mL volumetric flask (now  $2.00 \times 10^5$  ng/mL) into the 25 mL volumetric flask using a micropipette and subsequently place the stopper back into the 10 mL volumetric flask.
7. Using the deionized water in the beaker, fill the bulbous portion of the 25 mL volumetric flask by directly pouring the water from the beaker into the 25 mL volumetric flask until reaching the neck. Then, fill up to the mark on the 25 mL volumetric flask using a transfer pipette, ensuring the meniscus falls on the mark.

8. Stopper the 25 mL volumetric flask and twist to ensure a good seal. Place your thumb over the top of the stopper and invert the flask, so the air bubble rises to the top (bottom of the flask) and then turn the flask right side up. Invert the 25 mL volumetric flask with this process 20 times.
9. You now have a 25 mL solution of  $2.00 * 10^3$  ng/mL red 40. Before analyzing the solution with UV-vis spectroscopy at 503 nm, first the spectrophotometer must be calibrated.
10. Fill a cuvette approximately 80% full (3 mL) with deionized water, making sure to not overflow the cuvette. Avoid getting fingerprints on the sides of the cuvette.
11. Set the spectrophotometer to a fixed scan (Basic ATC on Genesys) at 503 nm and then measure the blank (place in B for Genesys). Ensure the cuvette is placed properly into the spectrophotometer with the clear walls of the cuvette facing the sides.
12. Remove the blank from the spectrophotometer.
13. Now that the spectrophotometer is calibrated using the blank, measure the absorbance of the  $2.00 * 10^3$  ng/mL red 40 solution at 503 nm. *Using transfer pipette* Fill a cuvette with 3 mL of the solution and measure the absorbance by placing the cuvette properly (place in B for Genesys) in the spectrophotometer and measuring at the calibrated 503 nm. Take 3 separate absorbance measurements for the  $2.00 * 10^3$  ng/mL red 40 solution. One measurement per cuvette, so you will measure the absorbance of 3 different cuvettes with 3 mL of the red 40 solution.
14. Record the absorbance measurements with the correct number of significant figures and you're done!

Exp. No.	2	Experiment/Subject	Quantitative Solution Prep and Serial Dilution	Date	1/30/25	004
Name	Lukas Rosipko	Lab Partner	Nathaniel White	Locker/ Desk No.		Course & Section No.

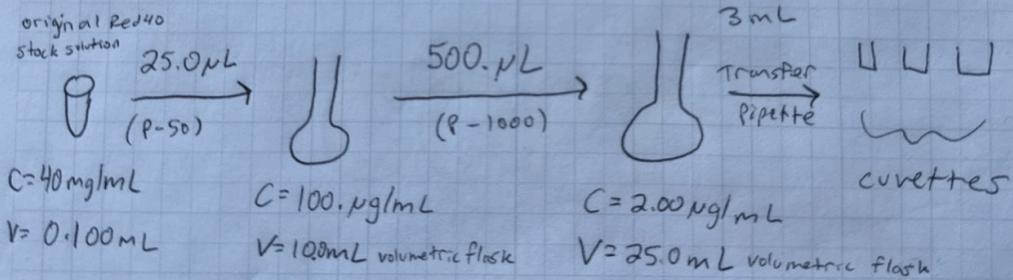
**Objective:** To create and share a SOP for the serial dilution of a red 4O solution. Further, to utilize UV-vis spectroscopy with Beer's law to confirm the concentration of the final solution.

**Safety:** Avoid consumption, breathing in, or getting any red 4O in your eyes. Ensure proper safety equipment is worn at all times.

**Procedure:** Following partners', Nathaniel white, SOP

- I chose to use a Fisherbrand Elite <sup>P-50</sup> micropipette S/N: MU44503
- I chose to use a 10mL Fisher Brand Volumetric flask

Model: FB-400-1D



- I chose to use a Fisherbrand Elite P-1000 S/N: PU07050

Blank: 0.000 Absorbance

Signature	Date	Witness/TA	Date
	1/30/25		

THE HAYDEN-McNEIL STUDENT LAB NOTEBOOK

Note: Place fold-over back cover under copy sheet before writing

Exp. No.	2	Experiment/Subject	Quantitative Solution Prep and Serial Dilution	Date	1/30/25	005
Name	Lukas Rosipko	Lab Partner	Nathaniel White	Locker/Desk No.		Course & Section No.

Trial #	Absorbance
1	0.082
2	0.082
3	0.082

Table: Absorbances of experimentally obtained  $2.00 \cdot 10^3 \text{ ng/mL}$  Red 40 solution

- I chose to use a thermoscientific Genesys 150 Model: GENESYS 1XX SW: 9ASZ189001

- Transferring approximately 3 mL solution to cuvettes, I chose to use Poltex disposable plastic transfer pipets

### Calculations

$$A_2 = C \cdot \epsilon_2 \cdot l$$

$$C = \frac{A_2}{\epsilon_2 \cdot l}$$

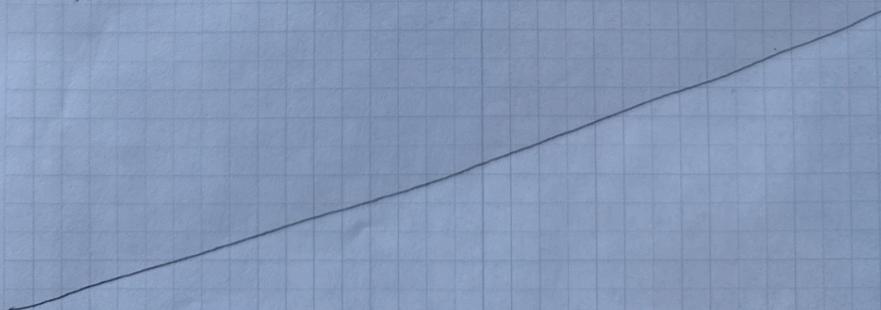
$$\begin{aligned} \epsilon_2 &= \epsilon_{503\text{nm}} = \\ A_2 &= A_{503\text{nm}} = 2.59 \cdot 10^4 \frac{\text{L}}{\text{mol} \cdot \text{cm}} \end{aligned}$$

$$l = 1 \text{ cm}$$

$$A_2 = A_{503\text{nm}} = 0.082 \quad \text{Same for all 3 trials}$$

$$C = \frac{0.082}{(2.59 \cdot 10^4 \frac{\text{L}}{\text{mol} \cdot \text{cm}}) \cdot 1 \text{ cm}} = 3.2 \cdot 10^{-6} \text{ M dilute Red 40 sample} \quad \text{Same for all 3 trials}$$

$$\bar{x} = 3.2 \cdot 10^{-6} \text{ M} \quad S_x = 0$$



Signature	Date	Witness/TA	Date
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2. The mean absorbance of the diluted solution is 0.083 with a standard deviation of 0.000.

The concentration of the final solution can be found as follows:

$$A_{503\text{ nm}} = c \epsilon_\lambda l$$

$$0.083 = c \times (2.59 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1})(1.0 \text{ cm})$$

$$c = \frac{0.083}{(2.59 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1})(1.0 \text{ cm})}$$

$$c = 3.2 \times 10^{-6} \text{ mol/L} = 3.2 \times 10^6 \text{ mol/L} \times \frac{496.42 \text{ g}}{1 \text{ mol}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = 0.0016 \text{ mg/mL}$$

The concentration of the initial solution can be found by reversing the serial dilution calculation:

$$0.0016 \text{ mg/mL} \times \frac{25 \text{ mL}}{0.25 \text{ mL}} \times \frac{10 \text{ mL}}{0.05 \text{ mL}} = 32 \text{ mg/mL} \pm 0.$$

This is significantly less than the expected concentration of 40.0 mg/mL, which is likely a result of operator error from filling a volumetric flask past the mark, or methodic error from water left from rinsing the volumetric flasks excessively diluting the sample.

3. This exercises involved the use of lab skills such as reading and writing SOPs, serial dilution, using a UV-Vis spectrophotometer, and performing calculations using Beer's Law.
4. This lab taught me about being specific about steps in a procedure, being more careful when dispensing with a transfer pipette, and about the fixed point mode of the UV-Vis spectrophotometer.
5. It appears that my partner was able to execute my SOP without issue. The critical comments he left related to specifying the need to avoid contaminating the stopper of the volumetric flask by setting it on the bench as well as a lack of detail with the instructions on how to use the spectrophotometer. He left an appreciative comment related to the diagram of the dilution scheme provided at the top of the SOP. In the future, I should include more detail on what to select in the menus of the spectrophotometer as he did on his SOP. It appears that either the sample or both of our SOPs are flawed since we both got similar absorbance values, with his absorbance values being  $0.082 \pm 0.000$ .

## Lab Notebook

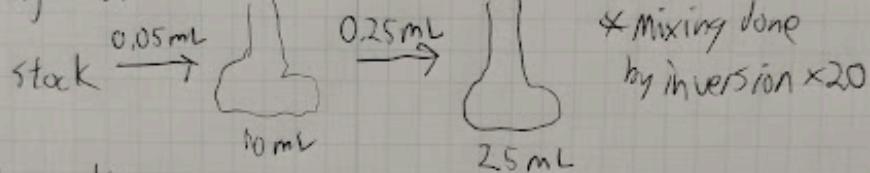
Exp. No.	2	Experiment/Subject	Quantitative Solution Prep. and Serial Dilution	Date	Jan 30
Name	Nathaniel White	Lab Partner	Lucas Rospito (procedure)	Locker/ Desk No.	Course & Section No. 2322

Objective: Obtain UV-Vis reading for the absorbance of a diluted solution of Red 40

UV-Vis Measurements  
0.083  
0.083  
0.083

Procedure:

Dilute from 40.0 mg/mL to  $2 \times 10^3$  mg/mL by way of



Used a thermo genesys (50 UV-Vis on fire) @ 503 nm  
Made to obtain three readings for three different cuvettes drawn from the final dilute solution. Results  
UV-Vis was blanked before starting measurements