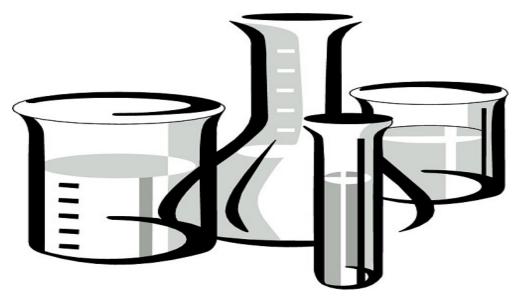


**College of the Canyons: Introduction to Biotechnology: Custom Lab: Solution Chemistry** 



Version 8-8-12

- Most biotechnology applications focus on the use of solutions to achieve a desired outcome.
- Solution chemistry traditionally uses the "mole" as a basic unit of solute concentration. From this mole, molar solutions are prepared when the solute is put into a solvent. The mole concept is dealt with more extensively in the Dimensional Analysis Lab.
- While useful, molar calculations can be cumbersome. So many alternative ways to define a solution's composition have been developed by biotechnologist including percent and parts.
- To be well versed in all of the solutions one may see in biotechnology lab, a review of the science of these calculations is a good idea.
- Complete all of the example calculations in the space provided, check your answers against the provided key and KEEP THIS LAB as a handy reference for both future biotechnology labs and perhaps other labs requiring an awareness of novel solution chemistry concepts..

For more information on College of the Canyons' Introduction Biotechnology course, contact Jim Wolf, Professor of Biology/Biotechnology at (661) 362-3092 or email: jim.wolf@canyons.edu. Online versions available @ www.canyons.edu/users/wolfj

These lab protocols can be reproduced for educational purposes only. They have been developed by Jim Wolf, and/or those individuals or agencies mentioned in the references.

**Please note:** This is a reference lab. You will not be handing in any aspect of this lab. Keep it handy during the entire semester, and return to it as needed (during quizzes, lab preparation, etc.). Each student in the course has their own level of comfort with the material. While some chemistry background is helpful, biotechnology has additional techniques (i.e. percent solutions, parts, etc.), terminology, etc., that any serious student of biotechnology should be aware of. This lab, the metric lab and the equipment lab should all be kept handy as they can help you with finding an item, making needed conversions and preparing needed media. You will notice some redundancy of the topics in this lab with other labs. Special care should be taking when citing units and preparing needed media. A periodic table (located at the end of the lab) can help you with the metric lab, making media, etc.

# **Overview:**

Solution preparation is a critical skill in a Biotechnology lab setting. The success of the lab is dependent on both correct solution concentration and preparation. Solution preparation involves basic laboratory procedures such as weighing compounds and measuring volumes of liquids. When preparing solutions you may also need to adjust the solution to a proper pH, sterilize it, or perform other manipulations. A solution must always be labeled with the following information: solution name, components (listed by amount), date of preparation, storage conditions, hazards and your full name (assuming you prepare it). A tracking number is usually included which allows for easier monitoring as well as cross-checking of all points alluded to on the complete label. The math used to calculate the solution composition is also sometimes included.

# Mass/Volume

The concentration of a solution can be expressed in many ways-most often in g/L or mg/L etc.

Concentration = <u>Mass of Solute(g or mg)</u> Volume of Solution(distilled water)

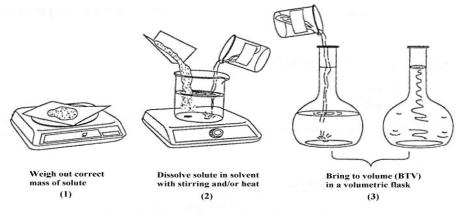
<u>Example:</u> Prepare a solution containing 3.5 g/L solution of CuSO<sub>4</sub>(aq). -(aq) indicates that the solute will be dissolved in an aqueous solution(H<sub>2</sub>O)

Step 1) Measure 3.5 g of CuSO<sub>4</sub>(s).

Step 2) Dissolve solute in less than 1 L of water.

Step 3) Fill volumetric flask to line to obtain desired volume and concentration.

**Bringing to Volume(BTV)**: this method of solution preparation involves adding solvent until the solution reaches its desired volume and concentration—which in our case is 1L. Volumetric flasks are usually used to prepare these solutions.



#### Figure 1. Basic steps for preparing a simple solution.

## I. Molarity

Another way to express the concentration of a solution is molarity. Molarity is used when the amount of solute is of greater importance than it's weight.

Molarity = <u>Moles of Solute(mol)</u> ←Always in "mols" Volume of Solution(L) ←Always Liters!

The word molarity, or molar, is abbreviated with an upper case M. It is also common in biology to speak of "millimolar" (mM) and "micromolar" ( $\mu M$ ) solutions.

Example:

1 M NaCl = 1 mole or 58.44 g of NaCl in 1 L of solution

1 mM NaCl = 1 *mmole* or 0.05844 g of NaCl in 1 L of solution

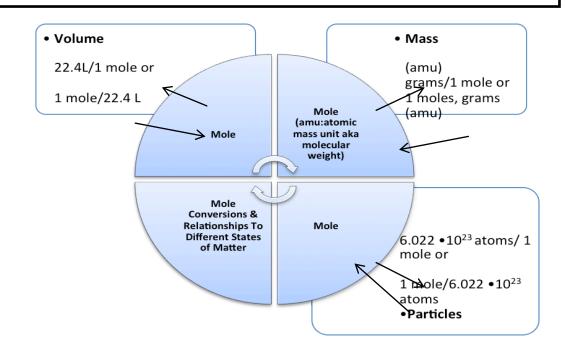
### What is a "mole"?

Just as a gram is a unit of mass, liter a unit of volume, second a unit of time, a mol is a unit of amount. A mole of any element always contains  $6.02 \times 10^{23}$  (which is known as the Avogardro's number) atoms. A mole of baseballs, ping pong balls and donuts all have  $6.02 \times 10^{23}$  of each item. The weight of a mole of a given element is equal to its atomic weight in grams, or its gram atomic weight. This information can be found on the periodic table, directly below the chemical symbol. The atomic weight of Carbon is 12.0 g. Because atoms of different

elements have different numbers of protons, neutrons, and to a lesser extent, electrons, a mole of one element weighs a different amount than a mole of another element. Carbon then has the weight of 12 grams per mole, oxygen 16 grams/mole, etc. The value of the mole is useful in that it allows one to use the atomic mass of an element and quickly convert this to a known number of atoms, molecules, etc.

When you are preparing a solution, your are usually given conditions under which a solution must have a specific concentration in a given amount of volume and is always in Liters. A problem would typically ask to solve for the amount of grams needed to prepare a solution so that a specific concentration is achieved. With that said you will need to know how to convert from moles to grams. Moles establish a connection between the three states of matter; gas, liquid, solid. This can easily be seen in the diagram provided below.

When converting from moles to any of the states of matter, the unit that is in the numerator is what you'll end with. On the other hand the unit that is in the denominator is usually what cancels out.



Example: Calculate the mass needed to prepare a 1L solution of 1.3 M LiBr?

Step 1) Determine how to solve the problem using dimensional analysis(what your given to start with and what you need to end with). So in this case we are given that we have 1 L of water, and need to end with a concentration of 1.3 M.

$$\begin{array}{ll} \text{Molarity} = \underline{\text{Moles of Solute}} & \rightarrow & 1.3 \text{ M or (m/L)} = \underline{\text{Moles of Solute(need to find )}} \\ \text{Volume of Solution(L)} & & 1L \end{array}$$

Step 2) Solve for the value needed(moles of solute).

-Using dimensional analysis, we want the units in the denominator to cancel(liters) so we multiply 1.3 m/L with 1L. Liters cancel out and now we have moles. We need exactly 1.3 moles of LiBr.

Step 3) Now that we have moles, we can convert to grams.

-First calculate the molar mass(g/mol) of LiBr. Occasionally the molar mass of the compound or chemical can be found on the bottle or internet.

 $Li^+= 6.94 \text{ g/mole}$  Atomic Mass of LiBr (g/mol) • mole = Grams of LiBr needed + Br<sup>-</sup>=79.9 g/mole 86.84 g/mole • 1.3 mol= 112.89 g LiBr needed 86.84 g/mole (mols cancel and your'e left with grams)

If the problem asks how many atoms are needed then you would use Avogadros constant  $(6.022 \cdot 10^{23})$ .

1.3 moles LiBr •  $\underline{6.022 \cdot 10^{23} \text{ atoms}} = 7.83 \cdot 10^{23} \text{ atoms of LiBr needed}$ 1 mole LiBr

We can determine how to prepare solutions of different molarities or different volumes by using straightforward proportional relationships.

Example: How much solute is required to produce 1 L of 0.25 M sodium chloride solution?

Using the reasoning of proportions, if a 1 *M* solution of NaCl requires 58.44 *g* of solute, then:

 $\underline{X}_{0.25 M} = \underline{58.44 g}_{1 M} \leftarrow \text{cross multiply and solve for X.} \qquad X(1) = 58.44(0.25)$ 

X = 14.61 g = amount of solute to make 1 L of 0.25 M NaCl.

It is sometime necessary to make more or less than 1 L of a given solution. In these cases, proportions can again be used to determine how much solute is required.

#### **II. Percents**

The are 3 ways to express percent concentration: mass per volume(m/v), volume percent(v/v), and mass percent(w/w).

#### A. Mass(weight) Per Volume

A weight per volume expression is the weight of the solute (in grams) per 100 mL of total solution. This is the most common way to express a percent concentration in biology manuals. If a procedure uses the term % without specifically stating otherwise, assume it is weight per volume (w/v).

Example: How would prepare a 500 mL of a 5% (w/v) solution of NaCl?

Step 1) Determine the percent strength and volume of solution required. Percent strength is 5% (w/v). Total volume required is 500 *mL*.

Step 2) Express the percent strength desired as a fraction (g/100 mL).

$$5\% = \frac{5 \text{ g solute}}{100 \text{ mL}}$$

Step 3) Multiply the total volume desired (Step 1) by the fraction in Step 2.

(5 g) . (500 mL) = 25 g amount of NaCl needed100 mL

Step 4) Bringing to Volume "BTV" Bring volume to 500 *mL*.

### **B.** Volume Percent (v/v)

In a percent by volume expression, abbreviated v/v, both the amount of solute and the total solution are expressed in volume units. This type of percent expression may be used when two compounds that are liquid at room temperature are being combined.

Volume percent is expressed as Milliliters of solute per 100 mL of solution.

Example: How would you make 500 mL of a 10% by volume solution of ethanol in water (v/v)?

Step 1) Determine the percent strength and volume required.

10% (v/v). total volume wanted is 500 mL

Step 2) Express the percent desired as a fraction (mL/100 mL).

 $10 \ mL/100 \ mL$ 

Step 3) Multiply the fraction from Step 2 by the total volume desired in Step 1 to get the volume of solute needed.

 $\frac{10 \text{ mL}}{100 \text{ mL}} \times \frac{500 \text{ mL}}{100 \text{ mL}} = 50 \text{ mL of ethanol needed}$ 

Step 4) Place the volume of the material desired in a graduated cylinder or volumetric flask. BTV (500 mL).

In summary...50 mL of ethanol in a 500 mL flask and BTV.

### C. Weight Percent (w/w)

Weight (mass) percent, w/w, is an expression of concentration in which the weight of solute is in the numerator and the weight of the total solution is in the denominator. This type of expression is uncommon in biology manuals. It is typically used for thick and viscous fluids like oil, paints, etc.

Weight percent is expressed as Grams of Solute per 100 grams of solution.

Example: 5 g of NaCl plus 20 g of water is 20% by mass solution: Weight of solute Total weight of solution

# **III.** Parts(ppm/ppb)

Solution parts tell you how many parts of each component to mix together. Parts can be expressed with respect to any unit of measurement(mass, volume, mols) as long as the units are consistent between all components of the mixture.

Example: Outline a preparation for a 24 mL solution that is 2:1:3 hexane : cyclopentane : ethyne.

First, we add the total number of parts required.

Here, we have 2 + 1 + 3 = 6 parts.

Next, we divide the total desired volume by the number of parts:

24 mL / 6 parts = 4 mL/part. Here, we have determined that every "part" in our solution will have a volume of 4 mL.

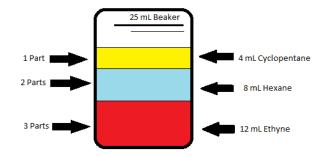
Thus the solution will require:

2 parts hexane =  $2 \times 4 \text{ mL}$  hexane = 8 mL hexane

1 part cyclopentane =  $1 \times 4 \text{ mL cyclopentane} = 4 \text{ mL cyclopentane}$ 

3 parts ethyne =  $3 \times 4 \text{ mL}$  ethyne = 12 mL ethyne

As expected, the total volume adds up to 24 mL.



## Parts per million(ppm) & Parts per billion(ppb)

-ppm & ppb are the expressions of "parts" most commonly used in Biology.

These expressions assume the following:

-Only two components are considered—solute and solvent.

-The solvent is water.

-"Parts" are with respect to mass.

ppm & ppb are expressed as:

(parts of solute) / (parts of solution)

Concentration is most often expressed in terms of ppm in environmental applications. This expression of concentration is useful when a very small amount of something (such as a pollutant) is dissolved in a large volume of solvent.

To prepare a 5 *ppm* solution in the laboratory, you must convert the term 5 *ppm* to a simple fraction expression such as milligrams per liter to determine how much of the solute to weigh out. Milligrams per liter, however, has units if weight in the numerator and volume in the denominator, but *ppm* and *ppb* expressions have the same units in the numerator and denominator. To get around this problem, convert the weight of the water into milliliters based on the conversion factor that 1 *mL* of pure water at 20°C weighs 1 *g*. For example:

#### For any solute:

1 ppm in water =  $\frac{1 \mu g}{mL}$  or  $\frac{1 mg}{l L}$ Also, 1 ppb in water =  $\frac{1 ng \text{ or } 1 ug}{mL}$  To make the expression simpler, it is possible to divide the numerator and the denominator both by 1 million:

5 ppm chlorine =  $5 \times 10^{-6} g$  chlorine (5 x 10<sup>-6</sup> g is the same thing as 5 ug) 1 mL water \*\*\* So, 5 ppm chlorine in water is the same as 5 ug of chlorine in water. 1.0 ml

<u>The derivation for these expressions is outlined as follows:</u> Having made the assumption that water is our solvent, and knowing that 1 mL of water has a mass of 1 g, we can set up the following equality:

 $1 \text{ ppm} = \underline{1 \text{ g solute}} = \underline{1 \text{ mg solute}} =$ 

Similarly, 1 ppb = <u>1 µg solute</u> <u>1 L of solution</u>

Taking "X" to be any arbitrary value, we can formalize this conversion as follows:

X ppm = <u>X mg solute</u>	$\mathbf{X} \mathbf{ppb} = \mathbf{X} \mathbf{\mu g} \mathbf{solute}$
1 L of solution	1 L of solution

Since ppm and ppb are derived units, we may use them as conversion factors.

### Ex: Outline a preparation for a 200 mL, 420 ppm solution of bromine (Br<sub>2</sub>).

First, we must determine the necessary amount of solute (Br<sub>2</sub>). We do so by using ppm as a conversion factor:

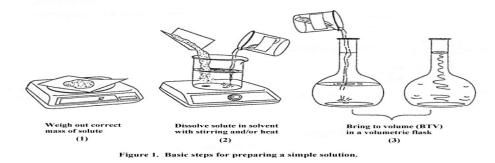
 $\frac{200 \text{ mL solution}}{1000 \text{ mL solution}} \times \frac{420 \text{ mg Br}_2}{1 \text{ L solution}} = 84 \text{ mg Br}_2 \text{ needed}$ 

Having performed the necessary calculations, we can now prepare the solution.

1. Weigh 84 mg of Br<sub>2</sub> into an appropriate container.

2. Add enough solvent (but less than the desired final volume) and mix thoroughly until the  $Br_2$  is dissolved.

3. Bring the solution up to volume by continually adding solvent until the solution reaches precisely 200 mL.



# IV. Molality & Normality

Molality(not to be confused with molarity) and Normality are expressions of concentration used far more in chemistry than in biology.

**Molality** is expressed as: (moles of solute) / (kg solvent) and is abbreviated with the unit lowercase "m"

For example, a 74.55 g sample of KCl (MW = 74.55) in 1 kg of water is a 1 m solution of KCl.

In solution preparation, the difference between a 1 M solution and a 1 m solution is as follows:

1 m: 1 mol of solute would be added to 1 kg of solvent.

1 M: solvent is added to 1 mol of solute until it is brought up to a final volume of 1 L.

**Normality** is expressed as (equivalent weight of solutes) / (liters of solution) and is abbreviated with the unit "N"

Biologists typically use normality when referring to strong acids and strong bases, so we will define normality with respect to these substances.

First, let us define equivalent weight.

**Equivalent weight** is the weight of solute required to produce 1 mole of a reactive group. For acids, the reactive group is H+ (hydronium ion). For bases, the reactive group is OH- (hydroxide ion).

Next, we will examine the nature of the following acids/bases, and determine their equivalent weight:

-1 mol of sodium hydroxide reacts with water to form 1 mol of hydroxide ion [ KOH $\rightarrow$  K+ + OH- ]

-Thus, the equivalent weight of KOH is simply the molar mass of KOH (56.11 g) -1 mol of sulfuric acid reacts with water to form 2 mols of hydronium ion [ H2SO4  $\rightarrow$  SO4-2 + 2H+ ]

-Thus, the equivalent weight of H2SO 4 is the mass of  $\frac{1}{2}$  a mole of H2SO 4 aka  $\frac{1}{2}$  the molar mass of H 2SO4 (49.1 g)

Lastly, we will outline the preparation of a solution using normality:

-To make a 1 N solution of KOH, we would weigh out 56.11 g KOH (aka 1 mole KOH) into the appropriate and add solvent until the solution is brought up to a final volume of 1 L.

Name\_\_\_\_\_ Date\_\_\_\_\_

# Concentration Expressions & Corresponding Calculations

Show all your work.

1. How would you prepare 50 mL solution of NaCl<sub>(aq)</sub> with concentration 0.3 g/mL?

2. You need to prepare a 1.5 g/mL solution of  $Ca(OH)_{2(aq)}$  using 3 grams of  $Ca(OH)_{2(s)}$ . What will be the total volume of your solution after it has been brought up to volume?

3. Assume that you have 6 L of 3 M  $Ca(NO_3)_2$ . How many mols of solute are there in this solution? How many grams?

4. What mass of solute is necessary to prepare a 500 mL solution of 4.20 M HC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>?

5. How would you prepare a 50 mL solution of 3 mM Na<sub>2</sub>CO<sub>3</sub>?

6. Suppose you have 25 g of LiBr (a crystalline solid) dissolved in 100 mL of water (density = 1 g / 1 mL).

a. Express this as a w/v % and as a w/w %

b. Explain why this concentration cannot be expressed as a v/v percent.

7. Determine the molarity of a 14% w/v solution of  $CuSO_4$ 

8. Suppose you have a 625 mL solution of 2: 1.2: 1.5 isopentyl alcohol : isopropyl alcohol : ethanol. What volumes (in mL) of each component exist in the solution?

9. How would you prepare a 30 ppb solution of Iron?

10. Convert 25 ppm to g / L

### III. Answer Key

### Practice Problems: Mass/Volume

### 1. How would you prepare 50 mL solution of NaCl<sub>(aq)</sub> with concentration 0.3 g/mL?

First, we need to determine the amount of solute necessary for the solution. Given the total volume of the solution (50 mL) we can use concentration as a conversion factor between volume of solution and mass of solute.

50 mL solution x 0.3 g NaCl = **15 g NaCl required** 1 mL solution (concentration)

Having performed the necessary calculations, we can now prepare the solution.

1. Weigh 15 g of NaCl into an appropriate container.

2. Add enough solvent (but less than the desired final volume) and mix thoroughly until the NaCl is dissolved.

3. Bring the solution up to volume by continually adding solvent until the solution reaches precisely 50 mL.

2. You need to prepare a 1.5 g/mL solution of  $Ca(OH)_{2(aq)}$  using 3 grams of  $Ca(OH)_{2(s)}$ . What will be the total volume of your solution after it has been brought up to volume?

Given the mass of Ca(OH)<sub>2</sub>, we can use concentration as a conversion factor from mass of solute to volume of solution.

 $\frac{3 \text{ g Ca(OH)}_2 \text{ x} 1 \text{ mL solution}}{1.5 \text{ g Ca(OH)}_2}$ (concentration)

In order to maintain a concentration of 1.5 g/mL and accommodate 3 grams of our solute, 2 mL of solution will be required when bringing the solution up to volume.

### **Practice Problems: Molarity**

<u>3. Assume that you have 6 L of 3 M Ca(NO<sub>3</sub>)<sub>2.</sub> How many mols of solute are there in this solution? How many grams?</u>

Given volume of the solution we can use molarity as a conversion factor from volume of the solution to mols of the solute:

<u>6 L solution x 3 mols  $Ca(NO_3)_2 = 18$  mols  $Ca(NO_3)_2$ </u> 1 L solution (molarity)

Given mols of solution we can use molar mass as a conversion factor from mols of solute to grams of solute:

Molar mass of Ca(NO<sub>3</sub>)<sub>2</sub>: 1 x Ca : 1 x (40.08) = 40.08 g/mol 2 x N : 2 x (14.01) = 28.02 g/mol <u>+ 6 x O : 6 x (16.00) = 96.00 g/mol</u> 164.1 g/mol

 $\frac{18 \text{ mols } Ca(NO_3)_2 x}{1 \text{ mol } Ca(NO_3)_2} = 2953.8 \text{ g } Ca(NO_3)_2$ 

```
4. What mass of solute is necessary to prepare a 500 mL solution of 4.20 M HC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>?
```

Using the volume of the solution (500 mL aka 0.5 L) we can use molarity and molar mass to convert from volume of solution to mols of solute, and from mols of solute to mass of solute.

Molar Mass of HC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>:

4 x H: 4 x (1.01) = 4.04 g/mol 2 x C: 2 x (12.01) = 24.02 g/mol + 2 x O: 2 x (16.00) = 32.00 g/mol 60.06 g/mol

5. How would you prepare a 50 mL solution of 3 mM Na<sub>2</sub>CO<sub>3</sub>?

First, we must determine the necessary amount of solute to prepare the solution. Given the volume of the solution (50 mL aka 0.05 L), we can use molarity and molar mass as a conversion factors to convert from volume of solution to mols of solute, and from mols of solute to grams of solute.

But before then, we will want to modify the given molarity (in mM) into something easier to work with (M), and calculate the molar mass of the solute.

3 mM Na<sub>2</sub>CO<sub>3 x</sub> <u>1 M Na<sub>2</sub>CO<sub>3</sub></u> = .003 M Na<sub>2</sub>CO<sub>3</sub> 1000 mM Na<sub>2</sub>CO<sub>3</sub>

Molar mass of Na<sub>2</sub>CO<sub>3</sub>: 2 x Na: 2 x 22.99 = 45.98 g/mol 1 x C: 1 x 12.01 = 12.01 g/mol + 3 x O: 3 x 16.00 = 48.00 g/mol 105.99 g/mol

 $\frac{0.05 \text{ L solution }_x 0.003 \text{ mol } \text{Na}_2\text{CO}_3 \text{ x } 105.99 \text{ g } \text{Na}_2\text{CO}_3 = .0159 \text{ g } \text{Na}_2\text{CO}_3}{1 \text{ L solution } 1 \text{ mol } \text{Na}_2\text{CO}_3}$ (Molarity) (Molar Mass)

### **Practice Problems: Parts**

<u>6. Suppose you have a 625 mL solution of 2 : 1.2 : 1.5 isopentyl alcohol : isopropyl alcohol : ethanol. What volumes (in mL) of each component exist in the solution?</u>

First, we sum the total number of parts in the mixture: 2 + 1.2 +1.5 = 4.7 parts Next, we divide the total volume by the number of parts: 625 mL / 4.7 parts = 132.98 mL per part Now we multiply each components number of parts with the above volume per part to obtain volumes of each as they exist in the solution: Isopentyl alcohol: 2 parts x 132.98 mL/part = **265.96 mL** Isopropyl alcohol: 1.2 part x 132.98 mL/part = **159.58 mL** Ethanol: 1.5 part x 132.98 mL/part =**199.47 mL** 

#### 7. How would you prepare a 30 ppb solution of Iron?

Using the definition of ppb, we have that 30 ppb is equal to  $30 \mu g$  solute per 1 L of solution. This is all the information we need to prepare this solution.

1. Weigh 30  $\mu$ g of Iron into an appropriate container.

2. Add enough solvent (but less than the desired final volume) and mix thoroughly until the Iron is dissolved.

3. Bring the solution up to volume by continually adding solvent until the solution reaches precisely 1 L of volume.

8. Convert 25 ppm to g / L

Using the definition of ppm, we have that 25 ppm is equal to 25 mg solute per 1 L of solution.

Using stoichiometry:

 $\frac{25 \text{ mg solute}_{x} 1 \text{ g of solute}_{z} = .025 \text{ g / L}}{1 \text{ L solution 1000 mg solute}}$ 

**Reference**: This lab was written by Eugene Lynch with guidance from Jim Wolf. Julian Barnes helped with formatting expertise.