Solution Making

One goal of the laboratory exercises in this course is for you to develop competence in laboratory procedures. Among the most fundamental of these procedures is preparation of solutions, and you will be required to prepare solutions for some of the lab exercises.

**Some general guidelines to follow when making solutions**

1. Always use ultra pure water (in plastic carboys) for solutions, unless indicated otherwise.

2. Dissolve substances in a volume of water less than the final volume (≈80%) needed. For example, you might start with 180 ml of water when preparing 200 ml of a solution. The solution will be brought up to the final volume after the pH is adjusted.

3. Know the pKa of the buffer being used. This is useful information when adjusting the pH; if the final pH of the solution is  ≥ 1 pH unit from the pKa, then you can expect the pH of the solution to change quite rapidly as you adjust the pH. The values can be obtained from the Merck Index, Sigma Chemical Catalog, and on-line.

4. As you adjust the pH, start with 10 M acid or base, as you approach the final pH, switch to a 1 M solution. How quickly the pH changes will depend upon the concentration of the buffer and how close the pH of the solution is to the pKa value. If you "overshoot" the final pH, you will need to start again.

5. Dissolve chemicals initially in a beaker on a magnetic stirrer. Measure final volumes with graduated cylinders; never use the gradations on beakers and flasks, since these are notoriously inaccurate.

6. Solutions should be stored in clean reagent bottles, labeled (on tape!) with your name, name of the solution and date. Any solutions containing proteins, carbohydrates or other labile components must be refrigerated.

**Cleanup**

You are expected to thoroughly clean all glass and plasticware that you use. These should be washed with soap and water, rinsed well with tap water, and finally rinsed inside and out with roH₂O at least 3 times. Glassware you used should be left on your bench to dry on paper towels.
Molar (M) vs. millimolar (mM) vs. micromolar (μM)

I assume that students in Biol 309 have had some experience using and calculating molar concentrations. You will need to use these skills this semester. In the field of cell biology, concentrations of substances are frequently given in mM, although μM and even nM (nanomolar) concentrations are often encountered. You need to be comfortable interchanging these units. The basic relationships are:

\[ 1 \text{ M} = 1000 \text{ mM} = 10^6 \mu\text{M} = 10^9 \text{nM} \]

For example: \( 0.02 \text{ M} = 20 \text{ mM NaCl} = 2 \times 10^4 \mu\text{M} = 2 \times 10^7 \text{nM} \)

There are practice problems dealing with units interconversions at the end of this section.

Calculating the amount of a dry reagent substance needed for molar concentration solutions

This general equation works for chemicals to be dissolved in M or mM concentrations

\[ \text{grams} = \left( \frac{\text{gram MW of chemical}}{\mu \text{ole}} \right) \times \left( \frac{\text{volume needed in Liters}}{1} \right) \times \left( \frac{\text{final concentration in Molarity}}{1000 \text{ mM}} \right) \]

Thus for example, if you need 150 ml of 50 mM glucose: (Glucose has a MW of 180)

\[ \text{Grams} = 180 \text{g/mole} \times 0.15 \text{ L} \times 0.05 \text{ M} = 1.35 \text{g glucose} \]

This is equivalent to:

\[ \frac{180 \text{g}}{\mu \text{ole}} \times \frac{150 \text{ml}}{1} \times \frac{1 \text{L}}{1000 \text{ml}} \times \frac{50 \text{mM}}{1} \times \frac{1 \text{M}}{1000 \text{mM}} \]

Percent (%) solutions

Sometimes the concentration of a component is given in percent. Units of “%(w/v)” (ie. percent weight/volume) give the weight of substance relative to the volume of solution, where 1%(w/v) = 1 g/100 ml. Thus, given a final concentration of a chemical (assuming you are starting with a pure, dry substance) and the required final volume,

\[ \text{gram of the chemical} = \text{Final volume (ml)} \times \text{fractional weight} \]

For example: to prepare 200 ml of 5% NaCl: 200 \times 0.05 = 10g of salt.

This would be prepared by dissolving 5g of NaCl in approximately 150 ml of water, and then bring the final volume up to 200 ml in a graduated cylinder.

If the component to be measured is a liquid, usually the units used are “%(v/v)” (i.e., percent volume/volume), where 1% = 1 ml per 100 ml. Thus, given a final concentration of a liquid chemical (assuming you are starting with a pure, 100%, substance) and the required final volume,

\[ \text{volume of the liquid chemical} = \text{Final volume (ml)} \times \text{fractional volume diluent} \]

For example, to prepare 200 ml of 70% ethanol: 200 \times 0.7 = 140 ml ETOH.

This would be prepared by adding 140 ml of ethanol to a graduated cylinder and then adding water to a final volume of 200 ml.
DILUTING SOLUTIONS

On occasion you will be confronted with the following type of problem:

"Prepare 5 ml of 15% mannose starting with a solution of 20% mannose."

The easiest way to calculate the necessary dilutions is to use the following relationship:

\[ V_1 \times C_1 = V_2 \times C_2 \]

where \( V \) equals the volume and \( C \) equals the concentration of the original (1) and final (2) solutions.

First, rearrange the equation to solve for \( V_1 \), and then plug in the known values:

\[ C_1 = 20\% \quad V_2 = 5.0 \text{ ml} \quad C_2 = 15\% \]

\[ V_1 = \frac{V_2 \times C_2}{C_1} = \frac{5.0 \text{ ml} \times 15\%}{20\%} = 3.75 \text{ ml} \]

The volume of water needed would be calculated as follows:

Final volume - volume of stock solution

\[ 5.0 \text{ ml} - 3.75 \text{ ml} = 1.25 \text{ ml H}_2\text{O} \]

Thus, you would mix 3.75 ml of 20% mannose and 1.25 ml of roH\(_2\)O to get 5 ml of 15% mannose.

<table>
<thead>
<tr>
<th>MWs of Some Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose (MW = 342)</td>
</tr>
<tr>
<td>Glycine (MW: use Google)</td>
</tr>
<tr>
<td>NaCl (MW = 58)</td>
</tr>
<tr>
<td>KCl  (MW = 74.5)</td>
</tr>
</tbody>
</table>
Practice Problems & Solution Making (20 points)

Show all calculations and use scientific notation where appropriate.
Always include leading zero (i.e. ‘0.34', not ‘.34')

Convert each of these concentrations to the indicated units:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Converted to</th>
<th>Concentration</th>
<th>Converted to</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 mM</td>
<td>_____ M</td>
<td>0.320 M</td>
<td>_____ mM</td>
</tr>
<tr>
<td>250 mM</td>
<td>_____ μM</td>
<td>0.005 μM</td>
<td>_____ mM</td>
</tr>
<tr>
<td>6 μM</td>
<td>_____ mM</td>
<td>500 μM</td>
<td>_____ M</td>
</tr>
</tbody>
</table>

Dilution Problems:

Which equation do you use for these problems:

1. How would you prepare 200 ml of 25 mM Tris-HCl starting with a 1 M solution.

2. Complete the following table showing how to prepare a series of 8 ml solutions, starting with a solution of 30% sucrose:

<table>
<thead>
<tr>
<th>Final [sugar]</th>
<th>ml of 30% sucrose</th>
<th>ml of H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>3%</td>
<td>_____</td>
<td>_____</td>
</tr>
<tr>
<td>7</td>
<td>_____</td>
<td>_____</td>
</tr>
<tr>
<td>11</td>
<td>_____</td>
<td>_____</td>
</tr>
<tr>
<td>15</td>
<td>_____</td>
<td>_____</td>
</tr>
</tbody>
</table>

3. How would you prepare 40 ml of 0.3 M NaCl starting with a stock solution of 1.5 M?

4. You have two stock solutions: 500 mM glycine and 0.1 M EDTA, and you wish to use these to prepare a 200 ml solution of 0.1 M glycine + 10 mM EDTA:

_____ ml of 500 mM glycine + _____ ml of 0.1 M EDTA + _____ ml H₂O
Percent solution problems:

Which equations described above would be used for these calculations?

How many grams are needed to prepare:

5. 50 ml of 3%(w/v) NaCl: _______

6. 1 liter of 25%(w/v) glycerol: _______

7. 100 ml of 30%(w/v) acrylamide: _______

mM solution problems:

Which equation do you use for these problems:

How many grams of each substance would be needed to prepare the indicated solutions?

8. 235 ml of 30 mM glycine: _______

9. 400 ml of 250 mM NaCl: _______

10. 800 ml of 10 mM NaH₂PO₄: _______

12. 20 ml of 100 mM glucose: _______

13. Suppose you had the following starting materials:
    a. 0.05 M solution of KCl    b. Trizma

    How much of each and water would you need to prepare 100 ml of a solution containing
    15 mM KCl and 125 mM Trizma?

    KCl: ______ ml   Trizma: ______ g   Water: _____ ml

★ Also complete and turn in the solution making section in the lab notebook. ★