1.9 Appendix -- Answer Key

1.8.1 Balances

1. Define precision. How is it related to accuracy? How does one measure precision?

Precision is a measure of how closely the measured values are grouped. Measurements with good precision have a tight or close grouping. Accuracy, on the other hand, is a measure of how closely the average value of the measurements is to the true value. Ideally we strive for good accuracy and good precision. It is possible to have poor accuracy and good precision, good accuracy and poor precision (luck), and poor accuracy and poor precision. We usually use standard deviation or a derivative of standard deviation to measure precision.

$$SD = \left[\sum (X - Xi)^2 / n - 1\right]^{1/2}$$

Where X = Average Value
Xi = Individual Value
n = Number of Runs

2. What is the USP definition of "accurately weighed" and how does it relate to "measurement uncertainty". How is the measurement uncertainty of a balance determined?

Accurate weighing is to be performed with a weighing device whose measurement uncertainty does not exceed 0.1% of the reading. We can determine the accuracy of a balance by comparing the balance reading with the stated value of the standard mass used. Measurement uncertainty is the combination of random and systematic error. Measurement uncertainty is satisfactory if three times the standard deviation (of not less than ten replicate weighings) divided by the amount weighed, does not exceed 0.001.

3. What do values of +/- 1 SD, of +/- 2 SD, and of +/- 3 SD tell us?

Measurements with a precision range of +/- 1 SD tells us that if the analyst continues to perform the measurements using exactly the same technique, then the results will fall within this range 68% of the time. +/- 2 SD = 95 % and +/- 3 SD = 98 %.

4. What is the relationship between SD and %RSD? What is the benefit of using %RSD?

%RSD = [SD / X] x 100 % where X is the average value. One benefit of %RSD is that the precision is normalized to a 100 point scale which allows us to readily compare precisions of two methods or analysts regardless of the analysis concentration range.

5. Describe two possible ways in which samples could be contaminated during weighing.

There are several possible contamination routes during weighing. An analyst could contaminate a sample during weighing by placing a contaminated spatula into the sample, by placing the sample on or into a contaminated holder during weighing, by dropping some lint/hair/skin or sneeze into the sample while weighing, or by opening up a bottle of chemicals near the sample being weighed. When performing trace analysis, it is possible for just a microgram of contaminant to be important, and a microgram is about 100 times less massive than a fingerprint!

6. What are some of the common errors in weighing and describe how to minimize them?

There are a number of possible ways that an error in weighing can occur. A few potential errors are:

- Misreading of the balance,
- Balance not level,
- Not cleaning the surface of the balance first,
- Touching the weighed object with moist hands,
- Leaving the balance doors open during weighing,
- Using a miscalibrated balance,
- Not cooling the sample down to near room temperature,
- Not removing a static charge from the sample,
- Excess vibration or air currents from people or nearby equipment, and
- Prolonged time sample left on pan adds/loses moisture.

1.8.2 pH Meters

1. Calculate the pH of a 0.075 M solution of acetic acid; assume the Ka = $1.8x10^{-5}$ for acetic acid. Prepare such a solution and measure the pH; calculate the % error and give at least three reasons for the error.

Let HA = Acetic Acid. $HA \leftrightarrow H^+ + A^-$ $1.8x10^{-5} = [H^+][A^-]/[HA]$
Let X = **M** of HA that dissociates at equilibrium.
$[H^+] = [A^-] = X$ $[HA] = 0.075\text{-}X \sim 0.075$
$1.8x10^{-5} = X^2/0.075$; $X = [H^+] = 1.2x10^{-3}$ **M**; pH = -log $[1.2x10^{-3}] = 2.9$

Possible reasons for error are: a) Errors involved in making the 0.075 **M** acetic acid solution from concentrated acetic acid. b) The concentrated acetic acid may not be 100 % acetic acid. c) The pH meter was not calibrated properly. d) Equilibrium calculations have at least a 10%

error due to the use of **M** instead of activity; activity depends upon ionic strength, concentration, and temperature.

2. Would a pH measurement in MeOH or in 50% MeOH - H_2O be accurate? Why or why not?

   No, the pH measurements would not be accurate. The surface of the indicator electrode needs to be coated with H_2O in order to function properly. Also the % ionization of the acid is dependent upon the water content.

3. Define buffer capacity.

   A buffer is a solution of a weak acid or a weak base and its salt which resists a change in pH. The capacity of the buffer to resist a change in pH when either a strong acid or a strong base is added is a measure of the buffer capacity. One definition of buffer capacity is: the amount of a given acid or base that can be added to the buffer solution in order to change the pH by one unit.

4. List several factors involved in selecting a buffer for a given procedure.

   The main factor is the pH -- a buffer only works if the pH is within one unit of the pK of the weak acid or weak base -- so select a buffer that has a pK as close to the pH as possible. Another factor is buffer solubility: for example, phosphate buffers may not be soluble in aqueous acetonitrile solutions. Other factors relate to buffer cost, buffer toxicity, buffer influence on microorganism growth, etc.

5. What is the useful range in buffering capacity about the pKa of the weak acid?

   pH = pKa +/- 1.0 A buffer only works if the pH is within one unit of the pKa of the weak acid.

6. Describe how one would prepare a liter of 0.0100 M phosphate buffer at pH = 8.00 using only 10.0 M H_3PO_4 and a concentrated NaOH solution.

   Add 1.00 mL of the 10.0 **M** H_3PO_4 to a little less than 1.00 L of water. Add enough concentrated NaOH to bring the pH to 8.00 (use calibrated pH meter) and then bring the volume to 1.00 L with water.

7. Discuss the use of an "equivalent weight" of an acid or base.

   The equivalent weight is the mass of the acid or base that will neutralize one mole of either H^+ or OH^-. The use of equivalent weight, equivalents, and the corresponding Normality (**N**) is useful since one equivalent of a substance will react with one equivalent of another substance; this simplifies calculations since the balancing coefficients of the reaction do not

enter into the calculation. The use of equivalent weight, equivalents, and the corresponding Normality (**N**), is an older concept and is frequently not taught in college chemistry classes.

8. What kinds of determinations, other than pH, may be made using a pH meter? Give two examples.

   One can use a pH meter as a potentiometer and measure the voltage developed at the electrodes in an oxidation-reduction reaction. Also, the pH meter can be used to measure the concentration of a given ion using an ion dedicated electrode.

1.8.3 Volumetric Glassware

1. Can all volumetric glassware be NIST certified?

   All volumetric glassware can be calibrated in the laboratory using accurately weighed deliveries of water since mass measurements can be more accurate than volume measurements (a chart of the densities of water at various temperatures is needed).

2. What do the letters "TC" and "TD" signify?

   The letters “TD” means “to deliver;” many volumetric pipets are calibrated to deliver a given volume after a given drain time. “TC” means “to contain;” many volumetric flasks are calibrated to contain a given volume.

3. Why should freshly boiled water be used in calibrating glassware?

   The density of the water does slightly depend upon the amount of dissolved gases, and boiling is a simple way to remove dissolved gases.

4. How would one know when a piece of volumetric glassware is not clean? What method would one use to clean it?

   The most common way to determine the cleanliness of volumetric glassware is to drain water from the apparatus; if the water drains smoothly and does not bead up, then it is considered clean. Volumetric glassware can become “dirty” just by picking up surface organics from the air. One way to clean the glassware is to rinse the glassware with chromic acid. Chromic acid is a solution of potassium dichromate in aqueous sulfuric acid. This solution is toxic, corrosive, and a strong oxidizing agent (caution); it will oxidize and dissolve most organics present. Other methods include rinsing with strong acid or base (depending on nature of deposit), rinsing with an organic solvent, or good old-fashioned scrubbing followed by a thorough rinse.

5. List the manufactures % accuracy of the following types of volumetric equipment: Class A 10 mL pipette, Class A 100 mL volumetric flask, 10 mL Mohr pipette, 1 mL Auto Pipette, 10 uL Auto Pipette, 100 mL graduated cylinder, 250 mL graduated beaker.

Note: The manufacturers' tolerances for Class A glassware may be better than the required standard. Class A volumetric glassware are those whose capacity tolerances are accepted by the National Bureau of Standards, and these tolerances are listed in the USP under Volumetric Apparatus [31]. The actual answers depend upon the manufacturer of the equipment; some possible answers are: Class A 10 mL pipet = 0.02 mL; Class A 100 mL Volumetric Flask = 0.08 mL; 10 mL Mohr pipet = 0.04 mL; 1 mL Auto Pipet = 0.02 mL; 10 uL Auto Pipet = 0.5 uL; 100 mL Graduated Cylinder = 0.5 mL; 250 Graduated Beaker = 10 mL.

6. List several of the variables involved in correctly using a 10 mL volumetric pipette.

Some variables involved in using a 10 mL volumetric pipet are: drain time; possible beads on the inner surface due to uncleanliness; temperature; bringing meniscus to the proper level; angle of drain; touching off last drop; rinsing of the pipet with the solution used; pipet calibration; etc.

1.8.4 Standard Solutions

1. What is a primary standard? What are its essential properties? Why does one dry the standard?

A primary standard is one which is weighed out and the concentration is known to be better than 1% accuracy. Some essential properties of the primary standard are: having a high purity; having a known purity; being stable under normal temperature and normal drying conditions; being stable under long storage conditions; and not being hygroscopic. Drying of the primary standard may be needed to bring it to a constant mass by removing any surface moisture.

2. What conditions are to be met to maximize the accuracy of a standardization?

The standards are to be of desired and known concentrations; the standard reacts quickly and completely with the reagents of interest; the instruments and glassware used allow the desired accuracy; the techniques used by the analyst are to be reproducible and allow the desired accuracy; and preferably, the weight of standard substance and volume of standard solution should not be too small. In general, titrations are made directly to the end point. A back-titration with another standardized solution increases the possibility of error. The standardization of a solution against another standardized liquid should be avoided. Every standardization should be based on at least three parallel determinations.

3. Define the normality factor and discuss its use.

Acid yields one, two, or three protons per formula. If the same number of formula weights of each, per liter, were dissolved the neutralizing capacities would not be the same. To express concentration in the terms of neutralizing capacity one does so according to the number of H_3O^+ or OH^- in the solution. A solution with one mole H_3O^+ or OH^- present for neutralization reactions is called "one normal solution." That weight of a compound or substance which will supply one mole H_3O^+ or OH^- for a neutralization reaction is called equivalent weight of the material. The equivalent weight of an acid is the formula weight divided by the number of protons per formula present for neutralization reaction.

4. Why couldn't one accurately weigh out solid NaOH to make the 0.1 N standard NaOH solution?

One can purchase a high and known purity NaOH product; so, why can't we just weigh out 4.00 g of 100% NaOH and dissolve to 1.00 L in order to make 1.00 L of a 0.100 **N** solution? The reason why this is not possible is that NaOH is highly hygroscopic; NaOH will rapidly absorb up to 100% of its mass in water from the atmosphere. Even if one has a pure sample of NaOH it will pick up significant amounts of moisture during a 30 second weighing.

5. Define a "1 in 2" solution; a "(1 + 2)" solution; a 10% W/W KI solution; a 10% V/V methanol - water solution; a 5% W/V acetic acid solution; a saturated solution. Calculate the normality and molarity of a 10% H_2SO_4 solution.

1 in 2 solution =1 volume liquid A + 1 volume liquid B (or sufficient volume of liquid B to make the volume of the finished solution two parts by volume). A *1 + 2 solution* = 1 volume of chemical A and 2 volumes of chemical B**.** A *10% W/W KI solution* = 10 g of KI added to 90 g of water. A *10% V/V methanol - water solution* = 10 mL methanol + enough water to make 100 mL total. A *5% W/V acetic acid solution* = 5 g of 100% acetic acid added to enough water to make 100 mL. A *saturated solution* = a solution containing the maximum amount of solute in solution, the undissolved solute in equilibrium with the solution.

M of a 10% H_2SO_4 (W/V) solution: (10g H_2SO_4 / 100 mL solution) x (1.0 mole H_2SO_4 / 98 g H_2SO_4) x (1000 mL / 1.0 L) **= 1.0 mole H_2SO_4 / L**. **N** of a 10% (W/V) H_2SO_4 solution: for H_2SO_4, **N** = **M** x 2 **= 2.0 equivalents H_2SO_4 / L**

1.8.5 Thermometers

1. Define partial immersion, total immersion, and complete immersion as related to thermometers.

A partial immersion thermometer has just its lower portion - the mercury bulb up to the marked immersion line - immersed in the sample. A total immersion thermometer involves immersion of the thermometer to the top of the mercury column, with the remainder of the stem and the upper expansion chamber exposed to ambient temperature. A complete immersion thermometer is entirely immersed in the sample.

2. How can an analyst recognize the different types (partial, total, or complete) of immersion thermometers?

The partial immersion thermometer is by far, the most common, and it will usually have an immersion line etched into it just above the mercury bulb; assume any unmarked thermometer will be a partial immersion type. The other two types will be plainly marked.

3. How does a maximum recording thermometer differ from a complete immersion thermometer?

A maximum recording thermometer only indicates the highest temperature achieved.

4. What is the best method for reuniting a separated mercury column?

The best way to reunite a separated mercury column is to place the thermometer into a very cold environment -- liquid N_2 or dry ice in acetone; allow the mercury to unite in the bulb; and then slowly warm back up to room temperature.

5. Why should an open flame not be applied to a thermometer to raise the temperature?

The intense heat from the flame will expand the thermometer rapidly and possibly crack the glass.

6. Why are two or more standards generally used to calibrate a thermometer?

If one calibrates a thermometer at a single temperature, then it will be accurate at that temperature; however, it could easily be off at other temperatures - especially at temperatures far from the calibration point. To help ensure that the thermometer is accurate over a selected range of temperatures, then one calibrates the thermometer at the lower portion of the range as well as at the upper portion of the range.

7. Why is the boiling point of pure H_2O generally not used as one of the two calibrating standards?

The boiling point of water depends upon the external pressure. For example, the BP of water at 760 Torr is 100 ^{o}C, and the BP of water at 630 Torr is 94 ^{o}C.

8. How many significant figures can be obtained from the temperature reading of a normal thermometer at room temperature?

   At a temperature of about 20 ^{o}C, a typical thermometer can be interpolated to 0.2 ^{o}C; so, three significant figures are possible.

9. List another typical device for measuring the temperature besides a glass thermometer.

   A thermocouple is a common temperature probe. It measures the temperature sensitive electric current flow through two dissimilar metals in contact with each other and converts the current to temperature.

1.8.6 Microscopes

1. Define the following types of illumination: bright field, dark ground, phase contrast, and fluorescence.

   Using bright field, object is illuminated with light. Using dark field, object reflects the light out of its surface and appears bright against a black background. In phase contrast microscopy, the unstained material can be viewed when light passes and is partially deflected by different thicknesses of the object. Fluorescence microscopy uses certain dyes. When the dye is excited by light of high energy (short wavelength UV), it emits the absorbed energy later as visible long wave light and the stained object is thus illuminated against a dark background.

2. Where is each form of illumination used?

   Bright field is used with stained objects and fresh material. Dark field is used with thin motile objects. Phase contrast is used with unstained material. Fluorescence is used for special diagnostic tasks like antibody-antigen diagnostic reactions.

3. What are the two ways one can tell an oil immersion lens from a high dry lens?

   It is marked 100X oil immersion lens. Its tip moves up and down.

4. List and describe each objective in the nosepiece.

   Framework: basic frame work includes arm and base.
   Lens system:
   a) Oculars: Two eyepieces, for the examiner to use to view object,
   b) the objectives usually three 10X, 45X and 100X, and

c) the condenser located under the stage, it collects and directs the light.
Nosepiece: to carry the lens system.
Light source: usually in the base of most microscopes.
Stage: horizontal platform that supports the microscope slide.

5. List and describe the ways the light source may be used to gain the best resolution.

   a) Change the wavelength of the light source by using filters. A blue filter should be used. This gives short wavelength and high resolution.
   b) Keep the condenser at its highest position, this allows maximum amount of light to enter the objective.
   c) Keep the diaphragm up, this increase the numerical aperture. (d) Use immersion oil with the 100 X lens.

6. List and describe the cleaning procedure for each microscope used in the laboratory.

   To be answered by individual instructor as requested.