

Basic Math & Chemistry for the Histology Laboratory

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Table of Contents

	page
Introduction	1
Math, Oh No!	
Chemistry is too hard!	
It's in the procedure	
Section 1.	2
Math, not just a four letter word	
Definitions	
Fractions, Decimals and Percent	
Conversions	
Word Problems	
Section 2.	8
Chemistry 101	
Definitions	
Units	
The Art of Weighing	
Solution preparation	
pH meters and calibrations	
What if? and other substitution problems	
Section 3.	17
Lab Safety	
Things that go boom in the night	
Don't lick your fingers	
Appendices	21
References	24
Answers to Problems (You thought I forgot, didn't you?)	25

Introduction

Do you panic when someone asks you to make a solution you've never made before? Does the thought of diluting a stock solution for immunohistochemistry give you the willies? Why? Perhaps you didn't take much math in high school or college. Maybe you think it's too hard. Basic math addition, subtraction, multiplication and division, are essential tools in the laboratory. Additionally, facility with fraction manipulation and Metric/ English conversion is essential for the production of quality solutions and therefore quality slides. These mathematic tools support and form the foundation of the chemical principles involved in the making of acids, bases, buffers, stains and other solutions. The following pages will review basic math principles, basic chemistry principles and general laboratory safety. The goal is to provide information and "real world" examples to take the mystery out of the math needed to produce chemical solutions in the histology laboratory. Each section will have problems to solve that involve the math and/or chemical principle covered. Answers appear in the back of the booklet (no cheating!) along with some valuable tables. Remember, math is a tool and chemistry is what we do every day in the histology laboratory. Everything you need to know may not be in the procedure. The tools provided here should help you prepare consistent solutions in the laboratory. Good luck.

Section 1

General Math

We're going to skip review of whole numbers, integers, negative numbers and imaginary numbers. We'll focus on those math skills that are of direct utility in the histology lab.

Definitions

Fractions: The mathematical representation for the consideration of a portion of a whole item consisting of a numerator and a denominator. The numerator (top number) represents the portion under discussion. The denominator is the total number of pieces that make up the whole item. Therefore: The mathematical symbol, $1/6$, numerically asks your consideration of 1 part of an item (real or imaginary) out of the 6 parts that comprise the whole item. Fractions may be added, subtracted, multiplied or divided following standard mathematical operation rules.

Percent: A special fraction where the denominator is understood to be 100 and the numerator is the given value followed by the percent symbol, %. Such as: 5 % acetic acid, meaning 5 parts of acetic acid in 100 (total) parts of solution.

Decimals: A special fraction where the denominator is understood to be a multiple of ten and the numerator is a portion of this whole part. Such as: $0.05 = 5/100 = 5 \%$

Operation rules: My Dear Aunt Sally and other rules

The order of arithmetic operations in the absence of parenthetical clues will proceed first with Multiplication, followed by Division, Addition then Subtraction. The order of operation when parentheses are present, proceed from the innermost set outward.

Fractions may only be added (or subtracted) together if the denominators have the same value. If the denominators are not the same, manipulate the fraction to produce the least common denominator prior to the addition or subtraction operation. Decimal representations provide the least common denominator in multiples of ten (by definition).

- $2/6 + 1/6 = 3/6$
- $6/20 - 2/20 = 4/20 = 2/10 = 1/5$
- $2/3 + 1/6 = 2 * (2/3) + 1/6 = 4/6 + 1/6 = 5/6$
- $5/12 - 2/5 = 5 * (5/12) - 12 * (2/5) = 25/60 - 24/60 = 1/60$
- $0.67 + 0.16 = 0.83$

Fractions may be multiplied together by keeping the numerators and denominators separate then reducing the resultant value to its least common denominator. When multiplying decimal numbers together, first multiply the numbers as if the decimals were not there. Then count the places held by the decimal in each number, add them together and mark the total number of places in the final value. In other words, consider the decimal as a fraction with the appropriate multiple of ten as its denominator. Then multiply as you would any fraction, reducing the answer to its least common denominator.

- $1/2 * 3/4 = (1*3)/(2*4) = 3/8$
- $5/7 * 2/6 = (5*2)/(7*6) = 10/42 = 5/21$
- $0.81 * 0.02 = (81 * 2)/(100 * 100) = 162 * 1/10000 = 0.0162$
- $1/5 * 0.68 = (1*68)/(5*100) = 68/500 = 17/125 = 0.136$

Division as a mathematical operation is the inverse of multiplication. Therefore division of fractions may be easily accomplished by using this unique relationship. When dividing one fraction by another the first step is to invert the divisor and change the operation sign from division to multiplication. Then multiply the fraction and reduce the answer to lowest terms.

- $1/2 / 3/4 = 1/2 * 4/3 = 4/6 = 2/3$
- $.06 / 1/3 = .06 * 3 = 0.18$
- $.68 / 5 = .68 * 1/5 = 0.136$
- $.42 / .07 = 6$
- $.28 / .56 = 28/100 * 100/56 = 28/56 = 1/2 = 0.5$

Scientific notation is a short hand representation of decimal fractions where the numerator is expressed as value between 1 and 10 and the denominator is expressed as 10^n , where n = a positive or negative whole number. Multiplication and division of values expressed in scientific notation may

be accomplished by performing the indicated operation to the leading values then adding (or subtracting) the exponents of the place holding base ten portion of the expression. Values in scientific notation may be written using E (Note: This E is capitalized not e lower case which is a representation for natural logarithms and their reciprocals) rather than 10 in the expression, e.g. $1.5 \times 10^6 = 1.5 \times 10^6 = 1,500,000$ or $1.5 \times 10^{-4} = 1.5 \times 10^{-4} = 0.00015$

- $0.045 = 4.5 \times 10^{-2}$
- $1254678 = 1.25 \times 10^7$
- $4.2 \times 10^6 / 2.1 \times 10^3 = (4.2 / 2.1) \times (10^6 / 10^3) = 2 \times 10^{(6-3)} = 2 \times 10^3$
- $5 \times 10^3 * 6 \times 10^3 = 3 \times 10^7$
- $6 \times 10^{10} / 3 \times 10^{11} = (6/3) * E(10-11) = 2 \times 10^{-1} = 0.2$

Metric/ English conversion:

Measurements in the laboratory as made using the metric system for weights, volumes, distance and temperature. We typically use grams, milliliters, microns and degrees Centigrade. But how do these measurements relate to each other and to the English system of ounces, pints, inches and degrees Fahrenheit?

Units of Weight:

Kilogram = 1000 grams = 2.2 pounds

(**Note:** At 1 atmosphere of pressure and 25 °C, 1 mL of water weighs 1 gram and will occupy 1 cubic centimeter (cc) of volume, therefore 1 L of water weighs 1 kg.)

Gram = 1000 milligrams

Milligram = 1000 micrograms

Pound = 16 ounces = 454 grams

Ton = 2000 pounds

Units of Volume:

Liter = 1000 milliliters

Milliliter = 1000 microliters

$1 \text{ cm}^3 = 1 \text{ mL}$ (of water at $25 \text{ }^\circ\text{C}$ and 1 atm)

Pint = 8 fluid ounces

Quart = 4 pints

Quart = 0.947 L = 947 mL

Gallon = 3.79 L

Units of Length:

Yard = 36 inches

Foot = 12 inches

Inch = 2.54 centimeters

Meter = 100 centimeters = 39.37 inches

Centimeter = 10 millimeters

Millimeter = 1000 microns

Micron (micrometer) = 1000 nanometers

Temperature:

$$F = 9/5 C + 32$$

$$C = (F - 32) * 5/9$$

Word Problems:

Nothing tends to cause those stomach acids to churn faster than the thought of doing word problems. But we're not trying to figure out where two trains will meet if they leave two separate points on the same rail line. We're trying to figure out how much acetic acid to use to make 500 mL of a 1 N solution. To solve these types of word problems, we need to figure out what we know (or where we can look it up) and what we need to produce. Solving word problems by identifying the units associated with the answer and those associated with the information given in the question can provide the path for the solution. For example:

- *How many grams are in 16 pounds? Express the answer in scientific notation.*

First: Identify the units associated with the question, in this example that unit is pounds.

Second: Identify the units associated with the desired answer, in this example, grams.

Third: What is the association between these units? In other words, what do you know about the relationship between these units? In this case, we know that 454 grams = 1 pound (see page 5 of this booklet). This means that when written as a fraction, 454 grams/ 1 pound has a value of one (or unity). Therefore: multiplying or dividing by this fraction will not alter the intrinsic value of the equation but allow you to convert the value from one unit of measure to another. This method of solving word problems is called the Unity Method.

Fourth: Arrange the question and answer units on opposite sides of an equal mark.

Question = Answer

Make a note of what you know (definitions that involve the units).

16 pounds = n grams, Known 454 grams = 1 pound or 454 grams/pound

Therefore:

16 pounds (454 grams/pound) = 7264 grams = 7.3×10^3 grams = 7.3×10^3 grams

Problems:

1. Express 25 degrees Centigrade (C) in Fahrenheit (F).
2. How many milliliters are there in 12 fluid ounces?
3. What is the weight in kilograms of 1 gallon of water? (Assume 1 atm pressure and room temperature).
4. Which volume is larger a quart of milk or a liter of Coke?
5. How many gallons are there in a swimming pool that is 15 feet wide, 30 feet long and 4 feet deep? (Given 1 gallon = 231 cubic inches).

Section 2

Basic Chemistry

In the histology laboratory we are asked to prepare solutions every day. Sometimes the instructions in the procedure are clear and describe in detail the process for making the desired solution. But often, the procedure assumes you know what a specific solution requires, where to get it and how much to use, e.g. 29 % ferric chloride, a component for the preparation of Weigert's Hematoxylin. Bancroft and Gamble (2002) kindly gives specific instructions for making this solution. I have been in labs where the Weigert's solutions were labeled "Weigert's A, 1 % hematoxylin" and "Weigert's B, 29 % ferric chloride" with the instructions to "mix 1:1 immediately prior to use." Although these instructions are true they are not entirely accurate and should a CAP inspector spy the bottles you'd be in violation. The point is that making solutions accurately cannot be taken for granted. Storage and handling of laboratory chemicals is an important part of our jobs. We're going to review in this section, basic chemistry definitions and principles and work a few word problems.

Definitions:

Mole: One mole of an element is defined to be the weight in grams equivalent to the atomic weight (at. wt.) of the element. *Example*: One mole of carbon weighs 12.011 grams. Similarly, one mole of a compound has a weight in grams equivalent to its molecular weight (abbreviated MW, sum of all the elements in the formula). Another term used for molecular weight is formula weight (abbreviated FW).

Molarity: The concentration of a solution in moles (molecular weight) per Liter. A one molar (1M) aqueous solution of sodium chloride (NaCl) is defined as one molecular weight (58.45 grams) of NaCl dissolved in one liter of water.

Normality: The concentration of a solution in equivalents per liter. An equivalent is defined as the number replaceable H⁺ or OH⁻ ions in an acid or base or as the number of exchangeable electrons in an oxidation-reduction reaction.

Equivalent = n X mole, where n = number of replaceable H⁺ or OH⁻ in an acid or base. Therefore for sulfuric acid (H₂SO₄), the equivalent weight = 2 X molecular weight which is 2 X 98 g/mole = 196 equivalents/mole.

Normality = n X Molarity, where n = the number of replaceable H⁺ or OH⁻ in the solution. Therefore, a one normal solution (1N) of sodium hydroxide contains one equivalent of OH⁻ for each mole of sodium hydroxide (MW 40). Since one equivalent weight of sodium hydroxide is equal to the molecular weight, a 1N solution = 1M solution of sodium hydroxide.

Percent (%) solution: Weight of substance (g) per 100 mL of solvent (w/v) or the volume of a solute (mL) per 100 mL of solvent (v/v).

Units and Formulas:

Mass or weight: Typically in grams or multiples of a gram.

$$1000 \text{ grams} = 1 \text{ kilogram} = 1 \times 10^3 \text{ grams}$$

$$1000 \text{ milligrams} (1 \times 10^{-3} \text{ grams}) = 1 \text{ gram}$$

$$1000 \text{ micrograms} (1 \times 10^{-6} \text{ grams}) = 1 \text{ milligram}$$

Volume: Typically in milliliters or multiples of milliliters.

$$1 \text{ Liter (L)} = 1000 \text{ milliliters (mL)} = 1 \times 10^3 \text{ mL}$$

$$1 \text{ mL} = 1000 \text{ microliters (}\mu\text{L)} = 1 \times 10^{-3} \text{ mL}$$

Dilutions: The volume of a stock solution of given concentration required to make a fixed volume of a more dilute solution can be calculated using the following relationship, $V_1 \times C_1 = V_2 \times C_2$. Where V_1 = the volume of the original concentration (C_1) required to make the desired volume (V_2) of the final concentration (C_2) needed.

- How many mL of 10 % NaOH do I need to make 100 mL of 1 % NaOH?

$$V_1 \times C_1 = V_2 \times C_2$$

$$V_1 \times 10 \% \text{ NaOH} = (100 \text{ mL})(1 \% \text{ NaOH})$$

$$V_1 = (100 \text{ mL}) (1\%) / (10\%)$$

$$V_1 = 10 \text{ mL}$$

Caution: The units associated with Volume must be the same and the units associated with Concentration must be the same.

Problems:

6. How many μL of 5 % NaOH do I need to make 10 mL of a 0.1N NaOH solution?
7. What is the molarity of 15 % solution of silver nitrate (FW 169.78)?
8. If concentrated sulfuric acid = 17.8 M, how much would I need to make a liter of 0.1 N solution?

The Art of Weighing:

Analytical balances are now available with digital displays and can weigh a wide range of masses from 0.00001 gram to several kilograms. You no longer have to slide weights of various sizes along beams of fixed length and align a mark with the balance point. However, the term balance still applies to the proper use of these digital wonders. To assure the accuracy of an analytical balance, it must be placed on a firm level surface. Periodically, the level of the balance should be checked using a small carpenter's level or a bubble level provided with the balance at purchase. Some balances have adjustable feet and may have a built-in bubble level. You should match the balance capacity to the items you need to weigh. For instance, don't weigh 1 mg on a top loading balance used to weigh 1000 gm Sprague-Dawley rats. Similarly, don't try to weigh a rat on an enclosed microgram balance. Use common sense.

Rules to live by:

- Calibrate your balances regularly. Quality assurance procedures for the lab require that the accuracy of the balances be certified annually. This may be done by trained lab personnel using standard weight sets or by an outside agency. Some of the newer digital balances have built-in calibration weights. These may be used for weekly calibration checks but **do not** substitute for annual calibration certification.
- Weigh onto paper or into an appropriately sized weight boat. Many of the chemicals we use are corrosive to the metal of the balance pan.
- Keep your balance clean. Not only do you not want to corrode your expensive balance, but who wants to ruin their solution by contaminating it with who knows what that was left on the balance!
- Use disposable wooden tongue depressors to scoop powders onto the weigh paper. These wooden sticks can be broken easily into narrow strips for handling small quantities of powder and can be

Solution Preparation:

You can't make a solution if you can't accurately measure liquid. Just as the proper use of a balance is critical in the accurate weighing of solids, so is the selection and proper use of cylinders and beakers for measuring liquids.

Containers come in a variety of shapes, sizes and precision. Erlenmeyer flasks and beakers are marked with approximate volumes but are not accurate enough to measure volumes for solution preparation. Measure the required volume in a graduated cylinder then pour it into a beaker or flask for mixing. For small volumes, 25 mL or less, use graduated pipets. For volumes less than 1 mL, use microliter pipets to measure volumes.

Rules and suggestions:

- Always add acid to water. For that matter, always add concentrated bases to water. NEVER add a concentrated base to an acid or vice versa. Always dilute them before mixing.
- For an accurate volume, read the bottom of the meniscus.
- When mixing solutions in graduated cylinders, let the solution rest on the bench for a few minutes to allow the meniscus to form before you top off the solution.
- Stay within the limits of your micropipets. Perform serial dilutions rather than skirting accuracy. For example, I need to make a 1:10,000 dilution of secondary antibody (2 mLs is enough). I can use a micropipet to measure 10 uL of stock antibody and dilute it with 990 uL of PBS. This gives me a 1:100 dilution. In order to make 2 mL of a 1:10,000 dilution, I can use 20 uL of the 1:100 dilution and add 1980 uL of PBS. (Remember, $V_1 \times C_1 = V_2 \times C_2$).
- Use a calibrated pH meter to adjust the pH of solutions. Stir the solution gently while adjusting and measuring the pH. Special caution, making and adjusting the pH of Tris buffers requires a particular type of pH probe. The tables supplied in appendix 2

shows how to make various pH Tris buffers without having to adjust the pH using a pH meter.

Table 1. Microliter pipets, volume ranges, Gilson pipetman style

Pipet	Range (μL)	Accuracy (μL)
P2	0.5 – 2.0	+/- 0.1
P10	1.0 – 10.0	+/- 0.1
P20	2.0-20.0	+/- 0.1
P200	10.0 – 200.0	+/- 1.0
P1000	100.0 – 1000.0	+/- 2.0

What if? And other substitution problems:

Let's see. I need to make a 5 % aqueous solution of Ferric chloride. That's five grams of Ferric chloride per 100 mL. Easy. I go to the chemical shelf and there are three bottles of Iron chloride there. One is labeled Iron (III) chloride, anhydrous. One is labeled Iron (III) chloride-6H₂O and the other is labeled Iron (II) chloride-4H₂O. Which one do I use? What if I don't have enough of the Iron (III) chloride, anhydrous? How much of the Iron (III) chloride, hexahydrate do I have to use for a 5 % solution? Can I use Iron (II) chloride-4H₂O?

These questions aren't as farfetched as they seem. Many times in the process of gathering the solutions for a procedure you'll run across substitution and dilution questions. In the above example, Iron (II) chloride [ferrous chloride] and Iron (III) chloride [ferric chloride] are two completely different chemicals and cannot be substituted for one another in most solution preparations. However, ferric chloride, anhydrous and ferric chloride hexahydrate can be substituted for one another in proportion to their molecular weights.

I need to make two different strengths of silver nitrate solution for a microwave Warthin-Starry stain, 2 % silver nitrate and 0.5 %. I've got some 5 % silver nitrate on the shelf from a Von Kossa stain. Can I dilute it and use it? No. Can I substitute potassium hydroxide for sodium hydroxide in a procedure?

Probably. Use common sense and know why you need the solution for the procedure.

In general:

- Make it fresh. Make the solution you need in the smallest volume possible. Label it completely, including the date made and the initials of the maker.
- Hydrated salts can usually substitute for anhydrous compounds in proportion to their molecular weight.
- In general, potassium chloride and sodium chloride can be freely substituted in proportion to their molecular weights. As can potassium hydroxide and sodium hydroxide. The proportionality caveat applies.

Problems:

12. How many grams of Alizarin red S (no purity given, FW 342.3) do I need to weigh to make 100 mL of a 2 % solution? What is the molarity of the solution?

13. What volume of concentrated HCl (12 M) do I need to make 60 mL of 2 N HCl?

14. How many grams of copper sulfate, anhydrous (99 % purity, FW 159.6) do I need to make 1 L of a 5 % solution? What is the molarity of the solution? How many grams of copper sulfate, pentahydrate (purity 98.0 – 102.9 %, FW 249.7) do I need to make an equal molar solution?

15. I need a solution containing 1 % non-fat dry milk in 0.1 N acetic acid. Glacial acetic acid is 17.4 M. Pretend I'm an idiot and tell me exactly how to make 250 mL of the solution.

Section 3

General Laboratory Safety

Why do we rarely consider laboratory safety until someone tells us a horror story or the CAP inspectors are due? Manufacturers have designed enclosed processors, vented coverslipping stations and bench-top formalin extractors. Nearly every lab has a chemical fume hood and flammable storage cabinet. So many safety measures are built into the design of laboratories that we tend to forget why they are there and why we should use them. Federal OSHA standards for permissible exposure levels for xylenes and formalin exist. Laboratories must prove they are in compliance with these standards. Lab workers are personally responsible for assuring their safety in the lab. Presented here is an overview of the chemical and biological hazards commonly found in the laboratory. Some general guidelines will be described for personal safety practices. Chemical storage recommendations will be presented with a glaring example of the consequences of failure to comply.

Biological & Chemical Hazards:

Tissues and body fluids are the major source of biohazards in the histology laboratory. However, biohazards are defined as anything that can cause illness in humans and includes chemical exposure. Lab workers are provided with personal protective equipment, gloves, eye shields and lab coats to prevent exposure to these hazards. But, how many of you have worn your lab coat to the cafeteria at lunchtime? Do you take off your gloves to answer the phone or write something down? Do you wipe up spilled blood immediately and treat the spill with bleach? Below are some of the commonly encountered biological and chemical hazards, excluding microorganisms of any kind.

Specific hazards: (See also www.osha.gov/SLTC/pel, Tables Z-1 & Z-2)

Formalin or formaldehyde: Classified as a carcinogen with a permissible exposure limit (PEL) -29 CFR 1910.1048(c)(1): "The employer shall assure that

no employee is exposed to an airborne concentration of formaldehyde which exceeds 0.75 parts formaldehyde per million parts of air (0.75 ppm) as an 8-hour TWA. Short-term exposure limit (STEL) 29 CFR 1910.1048(c)(2): "The employer shall assure that no employee is exposed to an airborne concentration of formaldehyde which exceeds two parts formaldehyde per million parts of air (2 ppm) as a 15-minute STEL."

Benzene and Toluene: Classified as carcinogens, Information from 29 CFR 1910.1000 Table Z-2

Substance	8-hour time weighted average	Acceptable ceiling concentration	Acceptable maximum peak above the acceptable ceiling concentration for an 8-hr shift	
			Concentration	Maximum duration
Benzene (a) (Z37.40-1969).....	10 ppm.....	25 ppm.....	50 ppm...	10 minutes.
Toluene (Z37.12-1967).....	200 ppm.....	300 ppm.....	500 ppm..	10 minutes

Other common laboratory reagents: From 29 CFR 1910.1000 Table Z-1:

Substance	CAS No. (c)	ppm (a) (1)	mg/m(3) (b) (1)	Skin designation
Acetic acid.....	64-19-7	10	25	
Acetone.....	67-64-1	1000	2400	
Ammonia.....	7664-41-7	50	35	
Benzoyl peroxide.....	94-36-0	5	
n-Butyl alcohol.....	71-36-3	100	300	
sec-Butyl alcohol.....	78-92-2	150	450	
Chloroform (Trichloromethane)...	67-66-3	(C) 50	(C) 240	
Ethyl alcohol (Ethanol)	64-17-5	1000	1900	
Formic acid.....	64-18-6	5	9	
Isopropyl alcohol.....	67-63-0	400	980	
Molybdenum (as Mo).....	7439-98-7			
Soluble compounds.....		5	
Nitric acid.....	7697-37-2	2	5	
Osmium tetroxide (as Os).....	20816-12-0	0.002	
Picric acid.....	88-89-1	0.1	X
Silver, metal and soluble compounds (as Ag).....	7440-22-4	0.01	
Xylenes (o-, m-, p-isomers)...	1330-20-7	100	435	

Chemical Storage and Incompatibilities:

Flammable chemicals must be stored in an approved flammable storage cabinet. Purchase and use the smallest possible volume of these chemicals. Sort chemicals into classes; organic chemicals vs. inorganic, dry vs. liquid. Salts of acids or bases should be stored in separate locations. Never store liquid acids or bases together or with dry chemicals or solvents. See Table 2 for specific incompatibilities.

In general:

- Organic liquids: Alcohols, xylenes, chloroform may be stored together in a flammable storage cabinet.
- Organic solids: Dyes and stains may be stored on the shelf away from acids, bases or salts of acids and bases. Tris base, urea and other organic solids may be stored with dyes and stains at room temperature. Seal the bottle with Parafilm™ after each use.
- Inorganic liquids: Store acids and bases separately at room temperature in an approved cabinet or under the chemical hood. Hydrogen peroxide (3 % solution and 30 % solution) may be stored in the refrigerator (4 °C) **but not with** organic liquids like alcohol or acetone!
- Inorganic solids: Store salts of acids separately from salts of bases at room temperature. Seal bottle with Parafilm™ after each use.

Table 2. Incompatible chemicals in the histology laboratory

Chemical	Incompatible with:
Acetic acid	Chromic acid, nitric acid, hydroxyl compounds, ethylene glycol, perchloric acid, peroxides, permanganates
Acetone	Concentrated nitric or sulfuric acid mixtures
Ammonia or Ammonium hydroxide	Mercury, chloride (gas, liquid or chloride salts), sodium or calcium hypochlorite, iodine, bromine, hydrofluoric acid
Ammonium nitrate	Acids, powdered metals, flammable liquids, chlorates, nitrites, sulfur, finely divided organic combustible materials (e.g. saw dust)
Chlorates	Ammonium salts, acids, powdered metals, sulfur, finely divided organic combustible materials
Chromic acid and chromium trioxide	Acetic acid, naphthalene, camphor, glycerol, alcohols, flammable liquids in general
Cyanides	Acids
Flammable liquids	Ammonium nitrate, chromic acid, hydrogen peroxide, nitric acid, sodium peroxide, halogens
Fluorine and fluoride salts	All other chemicals
Hydrocarbons (such as benzene, butane, alcohols)	Fluorine, chlorine, bromine, chromic acid, sodium peroxide
Nitrates	Acids
Nitric acid	Acetic acid, aniline, chromic acid, hydrocyanic acid, hydrogen sulfide, flammable liquids and gases, copper, brass, any heavy metal
Nitrites	Acids
Potassium permanganate	Glycerol, ethylene glycol, benzaldehyde, sulfuric acid
Silver (metal and salts)	Acetylene, oxalic acid, tartaric acid, ammonium compounds, fulminic acid
Sulfuric acid	Potassium chlorate, potassium perchlorate, potassium permanganate (or similar compounds of light metals e.g. sodium or lithium)

Appendix 1.

Properties of Concentrated Common Acids and Bases

<i>Acid or Base</i>	Molecular weight	% solution (w/w)	Specific Gravity (g/mL)	Molarity	Normality
Acetic acid	60.05	99.7	1.05	17.4	17.4
Ammonium hydroxide	35.05	28	0.90	14.8	14.8
Formic acid	46.03	97	1.22	25.7	25.7
Hydrochloric acid	36.46	37	1.20	12.2	12.1
Lactic acid	90.08	85	1.21	11.4	11.4
Nitric acid	63.01	70	1.40	15.5	15.5
Perchloric Acid	100.46	70	1.66	11.6	11.6
Phosphoric acid	98	85	1.69	14.7	44.1
Sulfuric acid	98.1	95	1.84	17.8	35.6

Appendix 2. Preparation of phosphate buffers.

To prepare phosphate buffer at any concentration (x molar) first prepare an x molar solution of dibasic salt using K_2HPO_4 or Na_2HPO_4 (solution A in the table below). Then prepare an equal molar solution of the monobasic salt using KH_2PO_4 or NaH_2PO_4 (solution B in the table below). Mix the solutions together in the proportion indicated check the resultant pH. Adjust pH with dilute acid or base as needed.

For 100 mL of x molar phosphate buffer:

pH	(mL) x molar HPO_4^{2-} Solution A	(mL) x molar H_2PO_4^- Solution B
6.3	24.0	76.0
6.5	33.4	66.6
6.7	44.3	55.7
6.8	50.0	50.0
6.9	55.7	44.3
7.0	61.3	38.7
7.2	71.5	28.5
7.4	79.9	20.1
7.5	83.4	16.6
7.8	90.9	9.1

Appendix 3. Preparation of Tris-HCl buffers.

Tris buffers can be made in two different ways depending on the chemicals you have on hand or wish to purchase. This is the method we use in our lab. Another scheme can be found in the SIGMA catalog (2002-2003 edition) on page 2092. To prepare Tris-HCl from Tris base and HCl, first prepare an x molar solution of Tris base (FW 121.14) labeled solution A in the table below. Then prepare an equal molar solution of HCl (aq. v/v). Mix the solutions together in the proportion indicated for the desired pH. Check the resultant pH and adjust as needed.

For 100 mL of x molar Tris-HCl buffer:

pH	(mL) x molar Tris base Solution A	(mL) x molar HCl Solution B
7.4	16.6	83.4
7.5	20.1	79.9
7.6	24.0	76.0
7.7	28.5	71.5
7.8	33.4	66.6
7.9	38.7	61.3
8.0	44.3	55.7
8.1	50.0	50.0
8.2	55.7	44.3
8.3	61.3	38.7
8.4	66.6	33.4
8.5	71.5	28.5
8.6	76.0	24.0
8.8	83.4	16.6

References

Web sites: www.iit.edu/~smile/index.html
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Answers to Problems:

Pre-test:

- 1 % solution is defined as containing 1 g of solute per 100 mL of solution. Therefore for a 5 % solution you'll need 5 g of the pure solute per 100 ml of solvent.
- $V_1 \times C_1 = V_2 \times C_2$, $V_1 = (1\%)(150 \text{ mL})/(15\%) = 10 \text{ mL}$, where
 - a. V_1 is the unknown
 - b. $C_1 = 15 \%$
 - c. $V_2 = 150 \text{ mL}$
 - d. $C_2 = 1 \%$
- 2N solution of KOH is equivalent to a 2 M solution and would contain by definition $2 \times (\text{FW})/\text{Liter}$. KOH FW = 56.11. Therefore a 2N solution would contain $2 \times 56.11 \text{ g/L}$ or 112.22 g/L. A percent solution is defined as grams of solute per 100 mL of solution. Therefore a 2 N solution of KOH is 11.22 %.
- Trick question! Look up table in appendix 3 on page 22 of the handout.
 - a. Make 0.1 Molar Tris base by dissolving 12.11 g/L of distilled water.
 - b. Make 0.1 Molar HCL by diluting 8.3 mL of the concentrated acid to a total volume of 1L.
 - c. For a liter of Tris buffer, pH 8.0: mix 443 mL of 0.1 M Tris base with 557 mL of 0.1 M HCl.

Problems:

- $F = 9/5 C + 32$, $F = 9/5 (25) + 32 = 9 \times 25/5 + 32 = 45 + 32 = 77 \text{ degrees}$
- Quart = 32 ounces = 947 mL, therefore $947 \text{ mL}/32 \text{ ounces} = 29.6 \text{ mL/ounce}$. Therefore, $12 \text{ ounces} \times 29.6 \text{ mL/ounce} = 355 \text{ mL}$
- Gallon = 3.79 L = 3.79 kg since 1 L of water weighs 1 kg at STP.
- As in three above, quart = 947 mL, 1 Liter = 1000 mL, therefore there is more coke than there is milk.
- $15 \text{ feet} \times 12 \text{ inches/foot} = 180 \text{ inches wide}$
 $30 \text{ feet} \times 12 \text{ inches/foot} = 360 \text{ inches length}$
 $4 \text{ feet} \times 12 \text{ inches/foot} = 48 \text{ inches deep}$
Volume = $L \times W \times D = 180 \times 360 \times 48 = 3,110,400 \text{ cubic inches}$
1 gallon of water is 231 cubic inches, Therefore, volume (gallons) = $3,110,400 \text{ cubic inches} / 231 \text{ cubic inches per gallon} = 13,465 \text{ gallons}$

6. $V_1 \times C_1 = V_2 \times C_2$, where $V_1 = (V_2 \times C_2)/C_1 = (10,000 \text{ uL} \times 0.1 \text{ N})/(1.25 \text{ N}) = 800 \text{ uL}$
- $V_1 = \text{volume in uL}$
 - $C_1 = 5 \% \text{ NaOH} = 1.25 \text{ N}$
 - $V_2 = 10 \text{ mL} \times 1000 \text{ uL/mL} = 10,000 \text{ uL}$
 - $C_2 = 0.1 \text{ N}$
7. 15 % silver nitrate = 15 g/100 mL or 150 g/L. Molarity = (wt (g)/ FW) per L = (150/169.78) per L = 0.88 N
8. Sulfuric acid 1 M = 2 N, $V_1 \times C_1 = V_2 \times C_2$, where $V_1 = (V_2 \times C_2)/C_1 = V_1 = (1000 \text{ mL} \times 0.1 \text{ N}) / (35.6 \text{ N}) = 2.8 \text{ mL}$
- $V_1 = \text{volume in mL}$
 - $C_1 = 35.6 \text{ N}$ since $M = 17.8$
 - $V_2 = 1 \text{ L} = 1000 \text{ mL}$
 - $C_2 = 0.1 \text{ N}$
9. Dye content = 50 % therefore need 2X weight of powder to yield 1X weight of dye. 250 mL needed * 5 mg/mL = 1,250 mg needed (pure) or 1.25 g pure dye, since dye content is 50 %, need to weigh 2.5 g of commercial powder.
10. 5 % = 5 g/100 mL = 2.5 g/50 mL, Molar ratio = 248.2 FW pentahydrate/158.2 FW anhydrous, purity is 99.5 %. Therefore to make 50 mL of 5 % $\text{Na}_2\text{S}_2\text{O}_3$ from the pentahydrate, weigh $2.5 \text{ g} \times (248.2/158.2) \times (1/0.995) = 2.5 \times 1.58 \times 1.005 = 3.97 \text{ g}$
11. Use analytical balance for the organ weight and top-loading balance for the body weight.
12. 2 g
13. $\text{HCl} 1 \text{ M} = 1 \text{ N}$, $V_1 \times C_1 = V_2 \times C_2$, where $V_1 = (V_2 \times C_2)/C_1 = V_1 = (60 \text{ mL} \times 2 \text{ N})/12 \text{ N} = 10 \text{ mL}$
- $V_1 = \text{volume in mL}$
 - $C_1 = 12 \text{ N}$
 - $V_2 = 60 \text{ mL}$
 - $C_2 = 2 \text{ N}$
14. CuSO_4 , 99 % pure therefore need 50.5 g for 1 L of 5 % solution. Molarity = 50.5 g / 159.9 GMW = 0.3 M. A 0.3M solution of pure pentahydrate = 0.3 M * FW per liter = 0.3 * 249.7 = 74.91 g per liter.
15. To prepare 250 mL of 0.1 N acetic acid, dilute 1.44 mL of glacial acetic acid into 250 mL total volume of deionized water. Weigh 2.5 g of non-fat dry milk and sprinkle it on top of the prepared acetic acid solution. Let set undisturbed until all the powdered milk dissolves. Mix gently and filter prior to use.

Post-test:

1. Use FeCl₂, anhydrous. Weigh 29 g and dissolve in 100 mL of 0.1 N HCl (aq.).
2. Collect 500 mL of distilled water in a graduated cylinder. Pour into a beaker. Transport the beaker to a chemical fume hood. Remove 2.87 mL of water using an appropriate pipet. Add 2.87 mL of glacial acetic acid and mix with stirring. Allow solution to cool to room temperature. Transfer to a 500 mL graduated cylinder and bring to 500 mL with an appropriate volume of dH₂O.
3. Stock concentration is 100 ug/mL for: 1 mL of diluted reagent containing the following
 - a. 0.5 ug/mL = 5 uL (100 ug/mL) + 995 uL diluent
 - b. 1.0 ug/mL = 10 uL (100 ug/mL) + 990 uL
 - c. 1.5 ug/mL = 15 uL (100 ug/mL) + 985 uL
 - d. 2.0 ug/mL = 20 uL (100 ug/mL) + 980 uL
4. Never store fluoride salts on the same shelf or in the same cabinet with ammonium salts. Ammonium salts readily react with moisture in the air to liberate ammonia (g). Ammonia (g) subsequently will react vigorously with fluoride salts making hydrofluoric acid which will etch glass and is quite toxic.
5. Use a P20 micropipette. Collect 10 uL of the stock antibody and dilute to 1000 uL total volume. This dilution represents 1:100 of the original. Then take 20 uL of the 1:100 solution and dilute to 2000 uL total volume. The resulting dilution is 1:10,000 of the original.