The ABC's and 123's of Laboratory Calculations

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Workshop Agenda

- **8:45 – 9:00:** Introduction and Welcome
- **9:00 – 10:00:** Part 1 – Review of Chemical Concepts
- **10:00 – 11:00:** Part 2 – Laboratory Practices and Calculations
- **11:00 – 11:45:** Part 3 – Statistics
- Q&A during each section

Introduction

Brief Resume

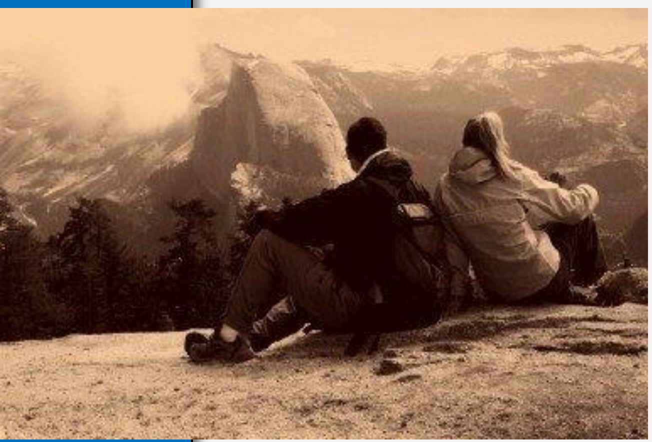
- BS Chemical Engineering
  - Washington University in St. Louis
- Project Engineer
  - ERM, St. Louis MO
- MS, PhD Environmental Engineering
  - Duke University
  - “Post-Doc” at NC State
- Assistant Professor
  - University of Massachusetts, Amherst
- Associate
  - Hazen and Sawyer, PC
- Professional Engineer
  - Massachusetts, Virginia

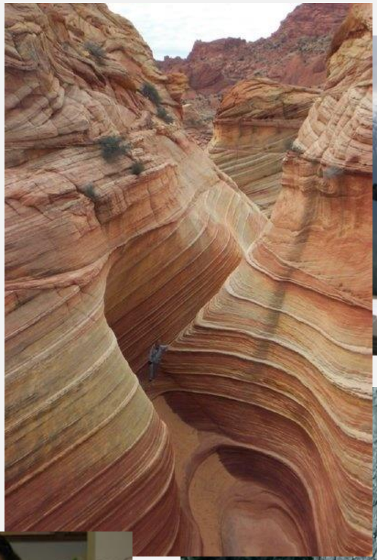
Introductions

- What I do professionally

Course Overview - Instructor

- Hobbies include running, hiking, basketball, playing with my wife and kids







Course Overview - Instructor

- Favorite Teams (in the interest of full disclosure)

Pitchers should HIT!!!

Course Overview - Instructor

- Least Favorite Teams

Introduction

- What we will go through today
  - Part 1: Review of Chemical Concepts
  - Part 2: Laboratory Practices and Calculations
  - Part 3: Statistics

- Part 1: Review of Chemical Concepts including:
  - The Periodic Table
  - Units of Measures (moles and equivalents)
  - Concentration (mass, molarity, and normality)
  - Acid/Base
  - Alkalinity and Hardness

- Part 2: Laboratory Practices and Calculations
  - Dimensional Analysis
  - Making Dilutions / Serial Dilutions
  - Making Standards from Stock Solutions
  - Titrations

- Part 3: Statistics
  - Precision and Accuracy
  - Significant Figures
  - Calculating RPD/RSD/% recovery
  - The theory behind correlation coefficients
  - Understanding the 95% Confidence Interval

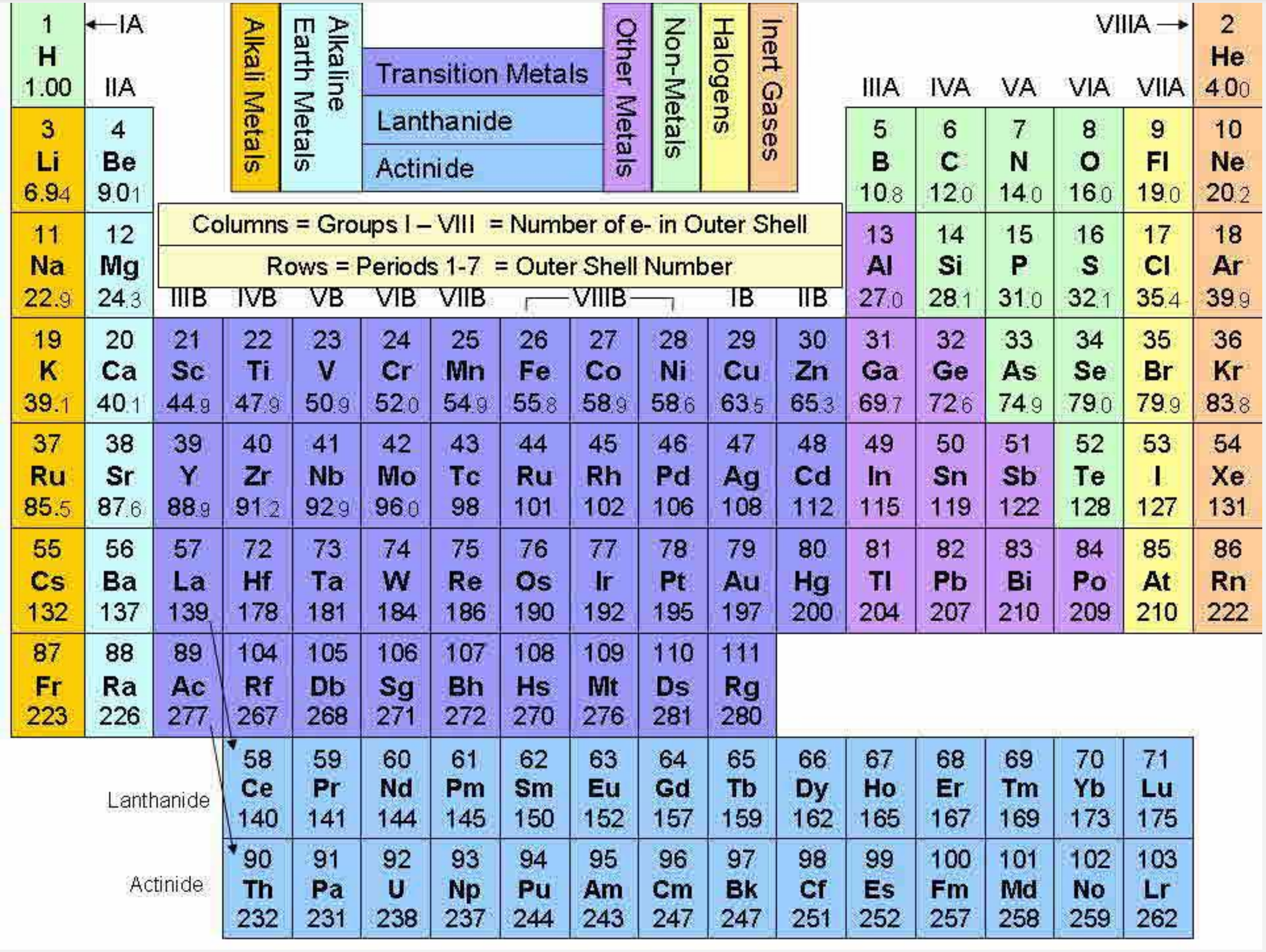
Example

- We will create our own data set and work with it:
  - Problem Statement:
    - Height and Wingspan in Human Beings has been observed to be linearly related. Let's see how that relationship holds in this workshop
  - Step 1: Measure every participants height and wingspan in inches (significant figures)?
  - Step 2: Convert height and wingspan to cm.
  - Step 3: Remember age
  - Step 4: Record on Data Sheet
  - Step 5: If you are #s 3, 6, 10, 12, 15 on the data sheet, repeat the measurements.

PART 1: REVIEW OF CHEMICAL CONCEPTS

The Periodic Table,
Units of Measures (moles and equivalents)
Concentration (mass, molarity, and normality)
Acid/Base
Alkalinity and Hardness

The Periodic Table



[We can learn a lot from the Periodic Table…]

Other examples of chemistry in action

http://www.youtube.com/watch?v=MTcgo46nxNE

http://www.youtube.com/watch?v=7Gp2wx2zlRI

One more Example:

- Chlorine + Ammonia
  - In drinking water = an effective disinfectant residual
  - In cleaning the bathroom = not good!
  - What's different between the situations?

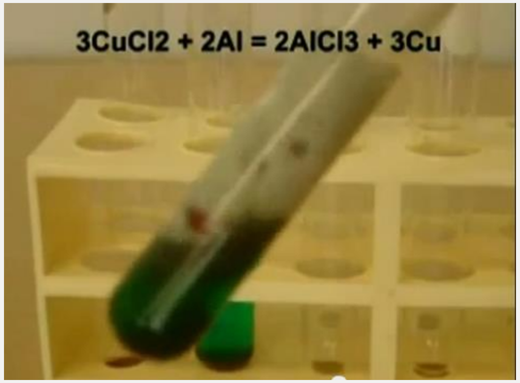
More Chemistry

Iron + Sulfur (+ Heat)

REDOX (Magnesium + Oxygen)

Potassium Chlorate + Sugar (+ Heat)

Combustion



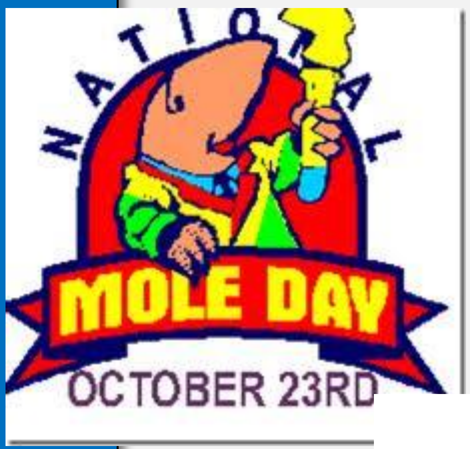
Copper Chloride + Aluminum

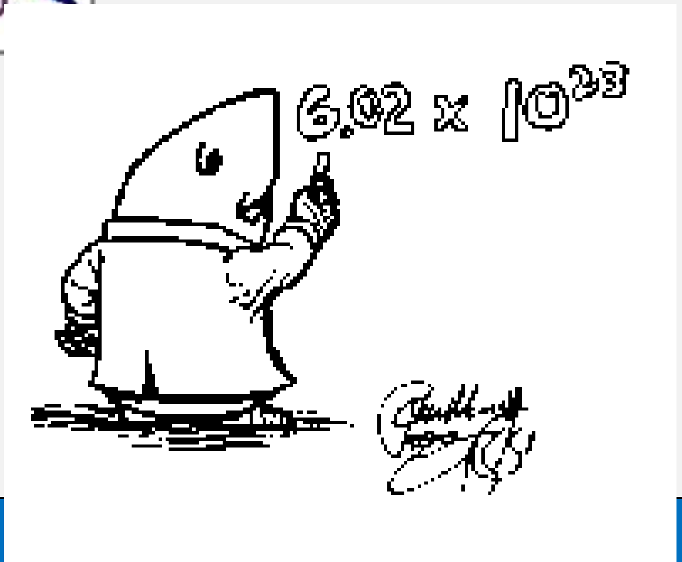
Guiding principal for today

- To understand laboratory calculations, and avoid “undesirable” outcomes, it is important to understand the terminology, equations, and fundamentals behind important calculations!!!

Chemical Concepts: The Mole

- "A day without mole calculations is like a day without sunshine!"





The Mole

- Unit of measure for comparing amounts of atoms and molecules
- 1 mol = 6.02×10^{23} atoms (or molecules)
- Mass of 1 mol = atomic (or molecular) weight
- Examples:
  - 1 mol carbon = 12 g
  - 1 mol CO_3^{2-} = 12 g + (16 g x 3) = 60 g

Equivalents

- The amount of a substance which will either:
  - react with or supply one mole of hydrogen ions (H^+) in an acid–base reaction; or
  - react with or supply one mole of electrons in a redox reaction.
- Examples
  - 1 mol of sulfuric acid (H_2SO_4) = 2 equivalents
    - 2 x H^+ supplied
  - 1 mol of Calcium Carbonate ($CaCO_3$) = 2 equivalents
    - CO_3^{-2} would consume 2 x H^+

Concentrations

- Mass Concentration = mass of a substance in 1L of solution (mg/L)
- Molar Concentration = Number of *moles* of a substance in 1L of solution (M, or moles/L)
- Normal Concentration = Number of *equivalents* of a substance in 1L of solution (N, or Eq/L)
- c(N) = a(M) x b(Eq/mol)

Concentrations

- Parts per million (ppm)
  - Parts of solute per million parts of total solution (mole per 1,000,000 mole, gram per 1,000,000 gram)
  - ie 30ppm KI = 30 grams of KI in 1,000,000 grams of water
- The water simplification
  - Density of water ➔ 1 kg = 1L
  - To the above example
    - 30 grams KI = 30,000 mg KI
    - 1,000,000 grams water = 1,000 L water
    - 30g KI / 1,000,000g water = 30,000mg KI / 1,000 L water
      - 30 mg/L water
- For WATER only, ppm = mg/L
  - Also, ppb = μg/L, ppt = ng/L, etc.

Implications: Water Pollutants

- The water and wastewater industry tends towards mass-based measurements (ppm, ppb, etc.)
- Molecular basis is often a more appropriate measurement
- Disinfection Byproducts (DBPs)
  - Total THMs = 80 µg/L
  - HAA5 = 60 µg/L
- THMs range from $CHCl_3$ to $CHBr_3$
  - 1 µmol $CHCl_3$ = 120 µg; 1 µmol $CHBr_3$ = 253 µg

Trichloromethane and Tribromomethane

- For trichloromethane:

$$80\ \mu g\ CHCl_3 \times \frac{1\ \mu mol\ CHCl_3}{120\ \mu g\ CHCl_3} = 0.67\ \mu mol\ CHCl_3$$

- For tribromomethane:

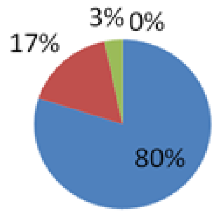
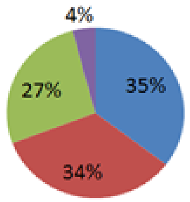
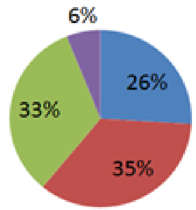
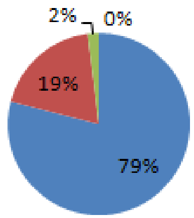
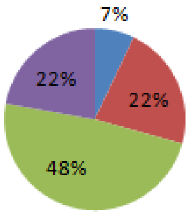
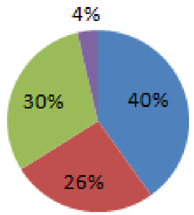
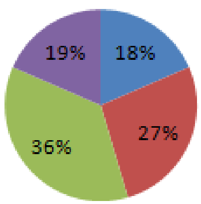
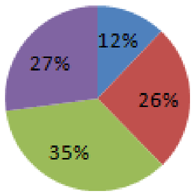
$$80\ \mu g\ CHBr_3 \times \frac{1\ \mu mol\ CHBr_3}{253\ \mu g\ CHBr_3} = 0.33\ \mu mol\ CHBr_3$$

- For a chloride-dominated water, potentially 2 x as many DBPs could be present on a molar basis!
- For systems dominated by brominated THMs it is harder to reach mass-based compliance!

DBP Example

- For the speciation charts on the next figure, show the differences in THM concentrations (mass basis) for the same molar concentration of 0.5 μM total THMs

DBP Example, Cont.

System	2004 - 2005	2007-2008	2012
System 1, VA			
System 2, NC		Chloroform Dichlorobromoform Chlorodibromoform Bromoform	
System 3, PA			

DBP Example, Cont.

- DBP Example Solution:
- System 2, NC
  - Step 1: mM of each component:

Component	2004 – 2005 Concentration (μM)	2012 Concentration (μM)
$CHCl_3$	0.395 (79%)	0.035 (7%)
$CHCl_2Br$	0.095 (19%)	0.11 (22%)
$CHClBr_2$	0.01 (2%)	0.24 (48%)
$CHBr_3$	0 (0%)	0.11 (22%)

  - Step 2: Convert to μg/L of each and sum

DBP Example Cont.

Component	Molecular Weight (g/mol)	2004 – 2005 Concentration (μg/L)	2012 Concentration (μg/L)
$CHCl_3$	119.2	0.395 x 119.2 = 47.1	0.035 x 119.2 = 4.2
$CHCl_2Br$	163.7	0.095 x 163.7 = 15.6	0.11 x 163.7 = 18.0
$CHClBr_2$	208.2	0.01 x 208.2 = 2.1	0.24 x 208.2 = 50.0
$CHBr_3$	252.7	0 x 252.7 = 0	0.11 x 252.7 = 27.8
		64.8	100.0

64.8 would be considered "acceptable", 100.0 is greater than MCL (80 μg/L)

Alkalinity Example

- Sweep Floc Coagulation with Ferric chloride
- $FeCl_3 + 3HCO_3^- \rightarrow Fe(OH)_3 + 3Cl^- + 3\ CO_2$
  - 3 moles of HCO_3^- are needed per mole of $FeCl_3$
    - 1 mole $FeCl_3$ = 161 mg $FeCl_3$ (neglecting H_2O)
    - 3 mole HCO_3^- (alkalinity) = 150 mg Alk as $CaCO_3$
- Question 1: If you add 40 mg/L $FeCl_3$, how much alkalinity do you consume (mmoles, mequivalents, mg)
- Question 2: If you have a 1N solution of sodium bicarbonate, how much do you need to add to provide enough alkalinity for a 40mg/L dose of $FeCl_3$

Alkalinity Example

- Question 1 Solution:
- $FeCl_3 + 3HCO_3^- \rightarrow Fe(OH)_3 + 3Cl^- + 3\ CO_2$

$$\frac{40\ \text{mg}\ FeCl_3}{\text{L}} \times \frac{1\ \text{mmol}\ FeCl_3}{162.1\ \text{mg}\ FeCl_3} = 0.246\ \text{mmol}\ FeCl_3$$

$$0.246\ \text{mmol}\ FeCl_3 \times \frac{3\ \text{mmol}\ HCO_3^-}{1\text{mmol}\ FeCl_3} = 0.74\ \text{mmol}\ HCO_3^-$$

$$0.74\ \text{mmol}\ HCO_3^- \ \frac{1\ \text{meq Alk as}\ CaCO_3}{2\ \text{meq}\ HCO_3^-} = 0.37\ \text{meq Alk as}\ CaCO_3$$

$$0.37\ \text{meq Alk as}\ CaCO_3 \times \frac{100\ \text{mg Alk as}\ CaCO_3}{1\ \text{meq Alk as}\ CaCO_3} = \frac{37\ \text{mg Alk as}\ CaO_3^-}{\text{L}}$$

Alkalinity Example

- Question 2 Solution:
- Assume 1 L of Solution

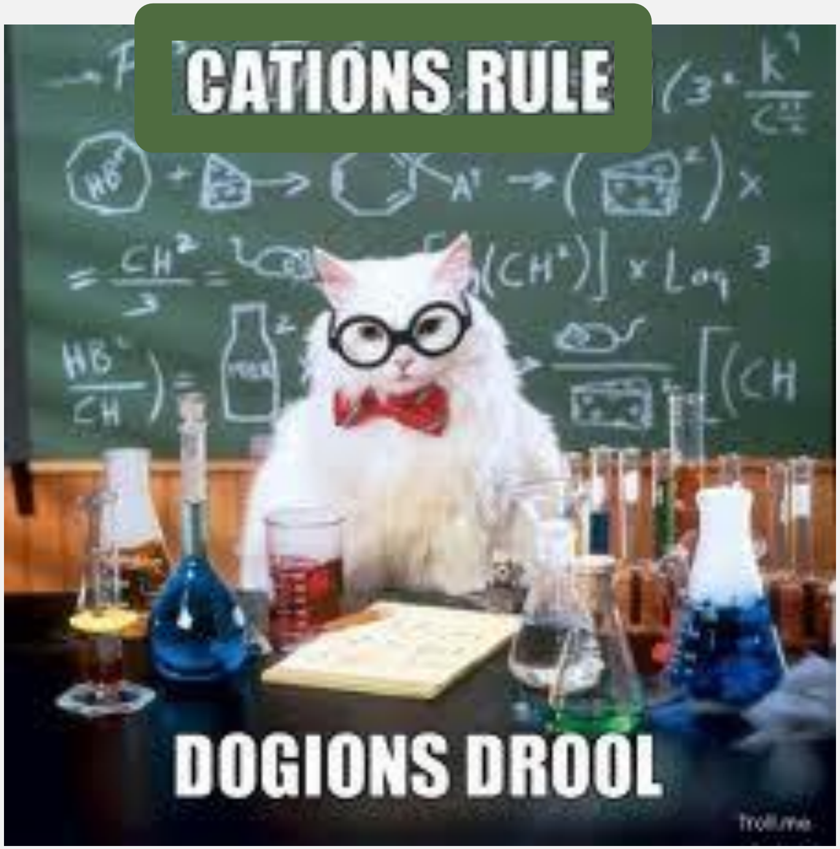
$$\frac{40 \text{ mg } FeCl_3}{L} \times \frac{1 \text{ mmol } FeCl_3}{162.1 \text{ mg } FeCl_3} \times 1L = 0.246 \text{ mmol } FeCl_3$$

$$0.246 \text{ mmol } FeCl_3 \times \frac{3 \text{ mmol } HCO_3^-}{1\text{mmol } FeCl_3} = 0.74 \text{ mmol } HCO_3^-$$

- $NaHCO_3 \rightarrow Na^+ + HCO_3^-$

$$0.74 \text{ mmol } HCO_3^- \times \frac{1 \text{ mmol } NaHCO_3}{1\text{mmol HCO3-}} \times \frac{1 \text{ eq } NaHCO_3}{1000 \text{ mmol } NaHCO_3} = 0.00074 \text{ eq } NaHCO_3$$

$$0.00074 \text{ eq } NaHCO_3 \times \frac{L}{1 \text{ eq } NaHCO_3} \times \frac{1000 \text{ mL}}{1 \text{ L}} = \frac{0.74 \text{ ml } NaHCO_3}{L \text{ solution}}$$

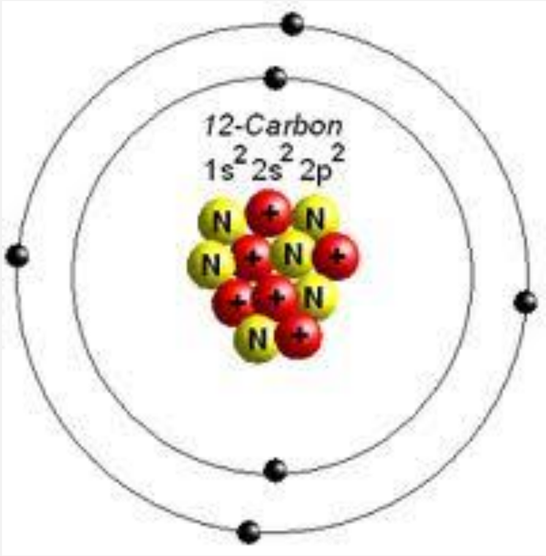

CATIONS RULE
DOGIONS DROOL

Acids & Bases

	$[H^+]$	pH	Common examples
Acids	1×10^{0}	0	Hydrochloric acid
	1×10^{-1}	1	Stomach acid
	1×10^{-2}	2	Lemon juice
	1×10^{-3}	3	Vinegar
	1×10^{-4}	4	Soda (carbonic acid)
	1×10^{-5}	5	Rainwater
	1×10^{-6}	6	Milk
Neutral	1×10^{-7}	7	Pure water
Bases	1×10^{-8}	8	Egg whites
	1×10^{-9}	9	Baking soda
	1×10^{-10}	10	Antacid
	1×10^{-11}	11	Ammonia
	1×10^{-12}	12	Quicklime (calcium hydroxide)
	1×10^{-13}	13	Drain cleaner
	1×10^{-14}	14	Lye (sodium hydroxide)

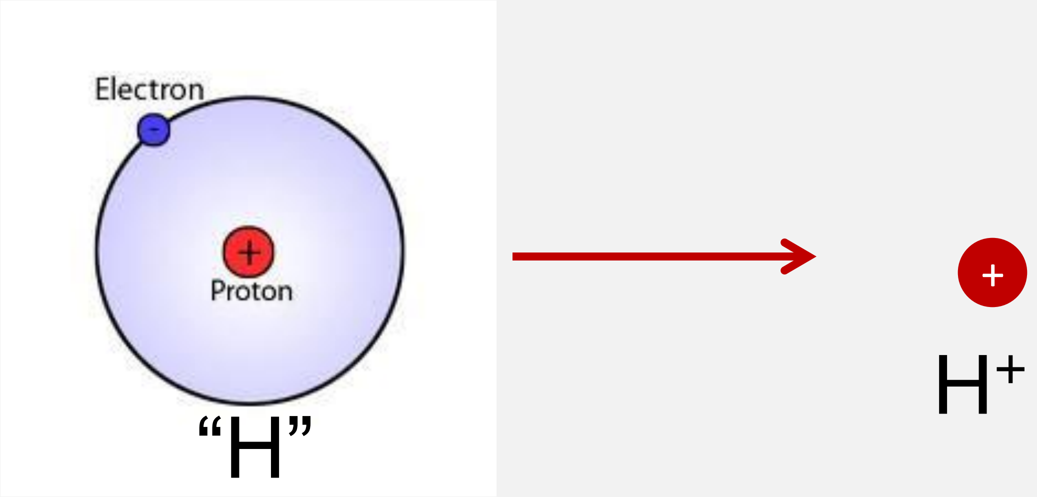
Acids & Bases

- First, about “protons”
  - Atoms = protons, neutrons, and electrons, right?
  - Carbon: Element 6, Atomic Mass = 12
    - 6 protons + 6 neutrons + 6 electrons



Acids & Bases

- First, about "protons"
  - Elemental hydrogen: diatomic, H_2
  - Alone, H has 1 proton and 1 electron
  - As a cation, H^+, the electron is absent, only the proton remains



Acids & Bases

- Acids and Acidity are generally associated with the availability of free protons (H^+) in solution
- Brønsted-Lowry Acid = Proton Donor
  - e.g., $HCl \rightarrow H^+ + Cl^-$
  - $HCl + H_2O \rightarrow H_3O^+ + Cl^-$
- Brønsted-Lowry Base = Proton Acceptor
  - e.g., $OH^- + H^+ \leftrightarrows H_2O$
  - $NaOH + HCl \rightarrow Na^+ + Cl^- + H_2O$
- Some species can be both acids and bases:
  - $HCO_3^- \leftrightarrows H^+ + CO_3^{2-}$
  - $HCO_3^- + H^+ \leftrightarrows H_2CO_3$
    - The behavior depends on the pH of the solution!

Strong Acids and Bases

- Strong acids and basis dissociate completely in water
  - NaOH → Na^+ + OH^-
  - H_2SO_4 → $2H^+$ + SO_4^{2-}
- Reactions “favor” right hand side
  - “Forward” reactions

Weak Acids and Bases

- Weak acids and bases dissociate incompletely
  - Some un-ionized fraction remains
  - $H_3PO_4 \leftrightarrows H^+ + H_2PO_4^-$
  - $H_2PO_4^- \leftrightarrows H^+ + HPO_4^{2-}$
  - $HPO_4^{2-} \leftrightarrows H^+ + PO_4^{3-}$
- Ratio of species based on equilibrium constant and pH
  - $K_{a1} = [H^+][H_2PO_4^-] / [H_3PO_4]$
  - Generically, $K_a = [H^+][A^-] / [HA]$

Concept of pH

- pH = -log[H^+]
- Each unit step down in pH is 10 x the hydrogen ion concentration!
- At pH = 7, [H^+] = [OH^-]

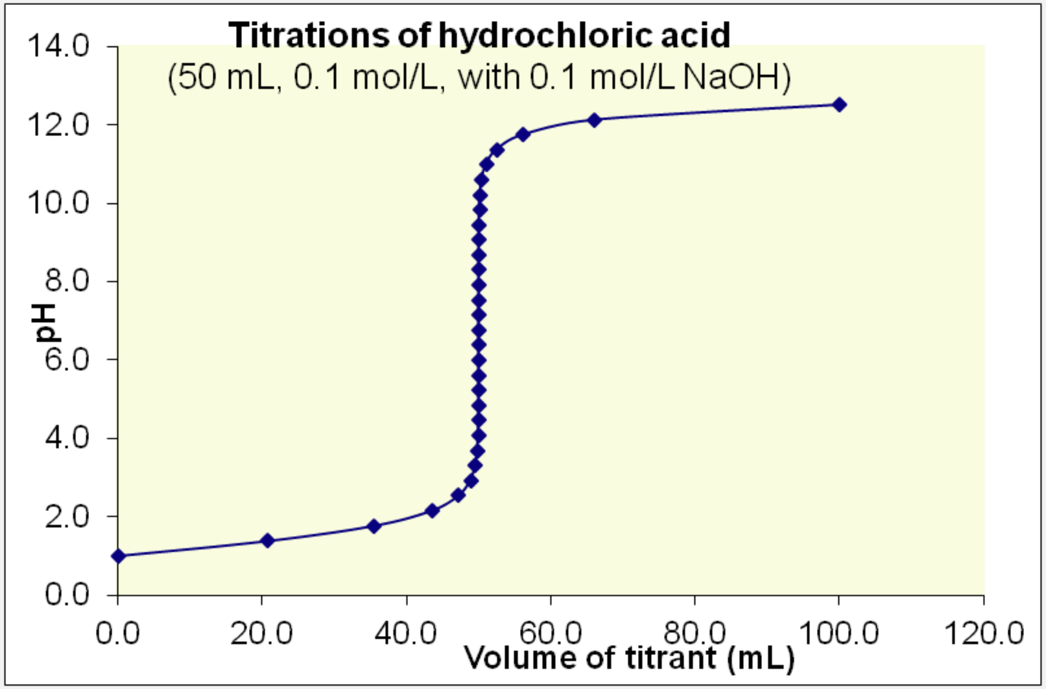
	[H^+]	pH	Common examples
Acids	1×10^{0}	0	Hydrochloric acid
	1×10^{-1}	1	Stomach acid
	1×10^{-2}	2	Lemon juice
	1×10^{-3}	3	Vinegar
	1×10^{-4}	4	Soda (carbonic acid)
	1×10^{-5}	5	Rainwater
	1×10^{-6}	6	Milk
Neutral	1×10^{-7}	7	Pure water
Bases	1×10^{-8}	8	Egg whites
	1×10^{-9}	9	Baking soda
	1×10^{-10}	10	Antacid
	1×10^{-11}	11	Ammonia
	1×10^{-12}	12	Quicklime (calcium hydroxide)
	1×10^{-13}	13	Drain cleaner
	1×10^{-14}	14	Lye (sodium hydroxide)

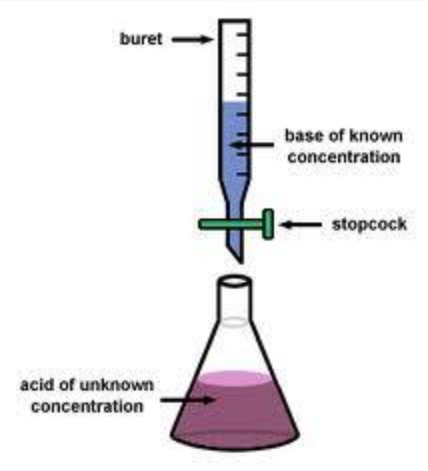
pH and pKa

- $pH = -\log[H^+]$
- And
- $K_a = [H^+][A^-] / [HA]$
- $K_a = [H^+] \times [A^-] / [HA]$
- $\log (K_a) = \log [H^+] + \log ([A^-] / [HA])$
- $-pK_a = -pH + \log ([A^-] / [HA])$
- $pH = pK_a + \log ([A^-] / [HA])$
- Thus, pH controls the amount of $[A^-]$ and $[HA]$ in solution

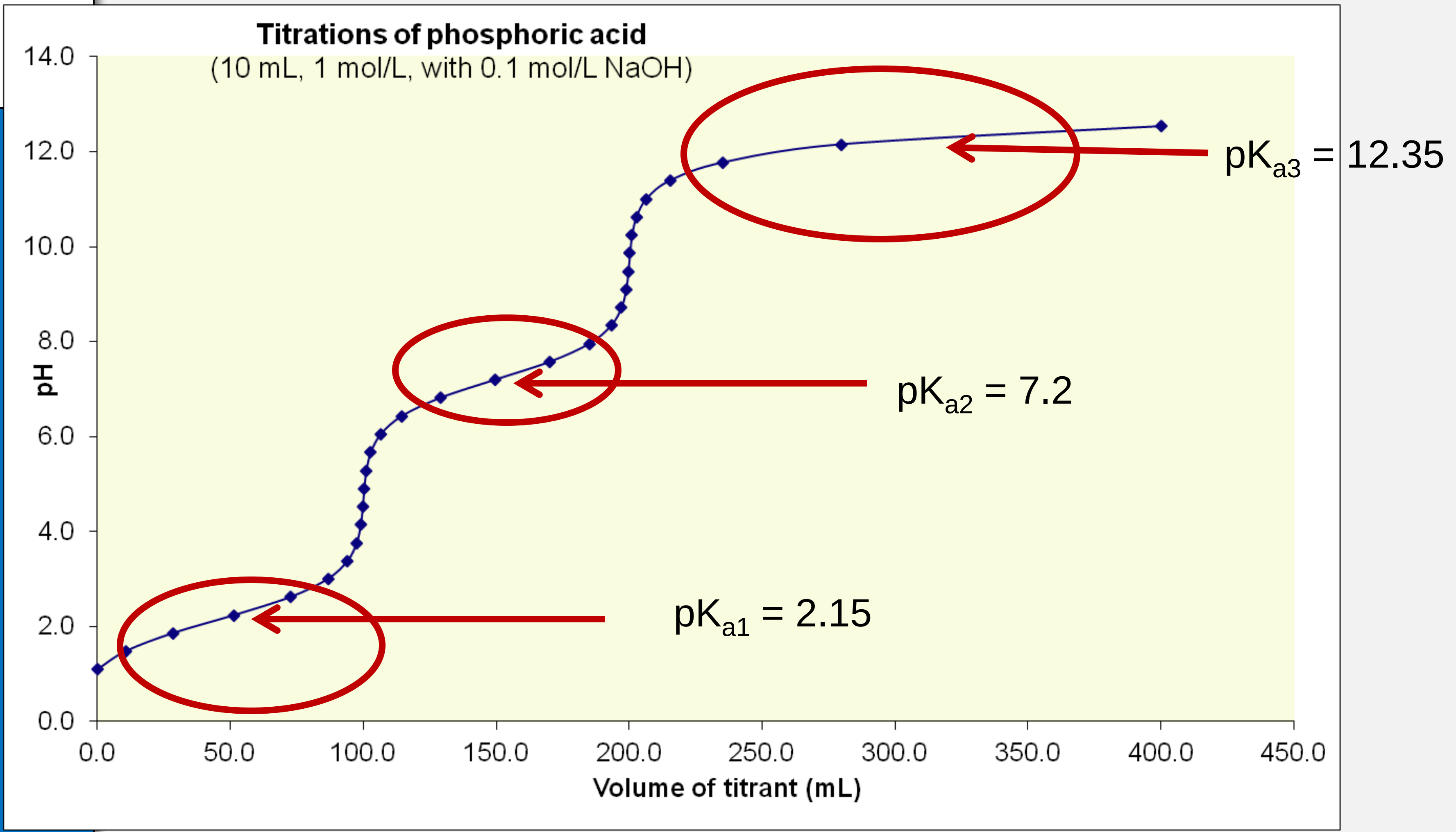
Titrations (Brief!!!!)

- Acids and bases will neutralize each other
- Titrations can be used to measure acids and bases in solution





Titrations Show pK_a Values and Buffer Capacity



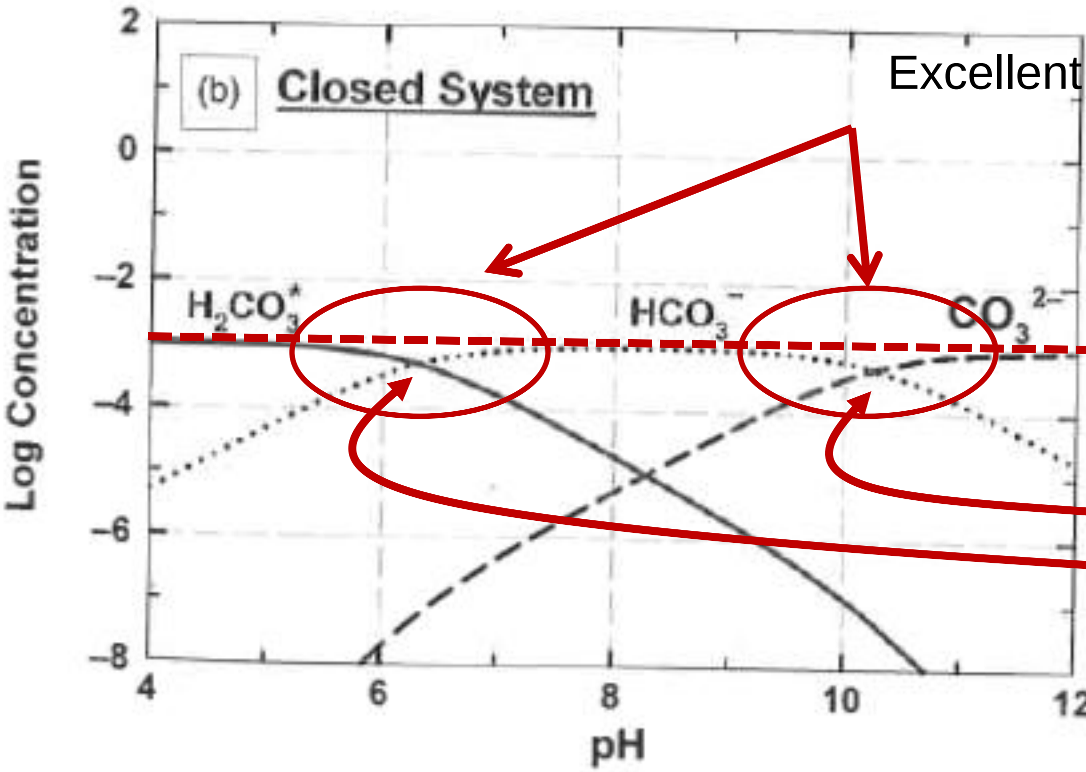
Buffer Capacity

- Regions that resist change in pH even when acids or bases are added
- Natural waters have different buffer capacities
  - Waters with high concentrations of divalent and trivalent ions tend to resist large shifts in pH
  - Deionized water will rapidly change pH when acids or bases are added

The Carbonate System, Closed

- One of the largest contributors to buffer capacity in natural waters is carbonate

Excellent Buffer Capacity!!



$C_{T,C} = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}] = 10^{-3}M$

$pK_{a2} = 10.33$

$pK_{a1} = 6.35$

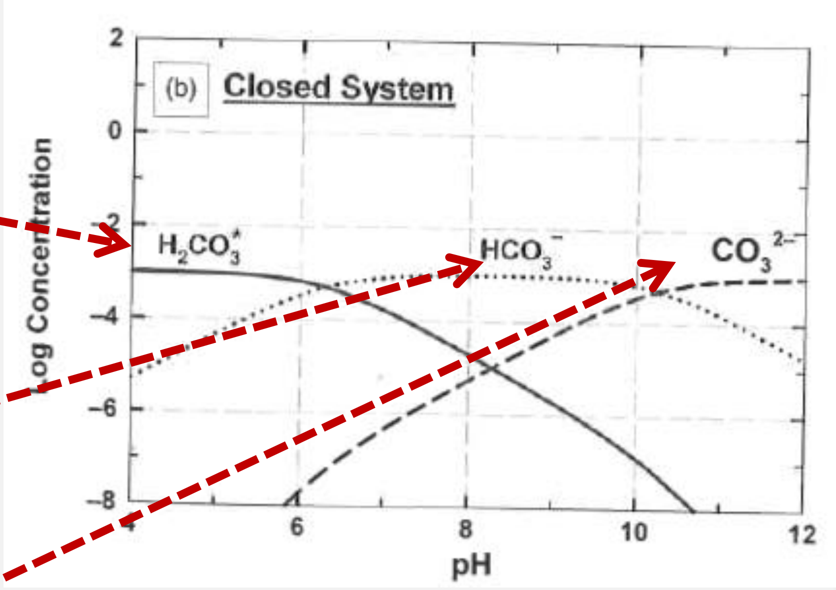
Calculating Carbonate Species

- Avoiding the details…
  - You can calculate the amount of each carbonate species at a given pH using alpha values which relate to acidity constants (K_a)
- $\alpha_0 = [H_2CO_3^*] / C_{T,C}$
- $\alpha_1 = [HCO_3^-] / C_{T,C}$
- $\alpha_2 = [CO_3^{2-}] / C_{T,C}$
- Tables are easier!!

Carbonate Alpha Values: Water Quality and Treatment Handbook

TABLE 3-8 Inorganic Acid-Base Alphas for K's at 25°C and Adjusted for 10^{-3} M Ionic Strength (pK_1 = 6.30 and pK_2 10.25)

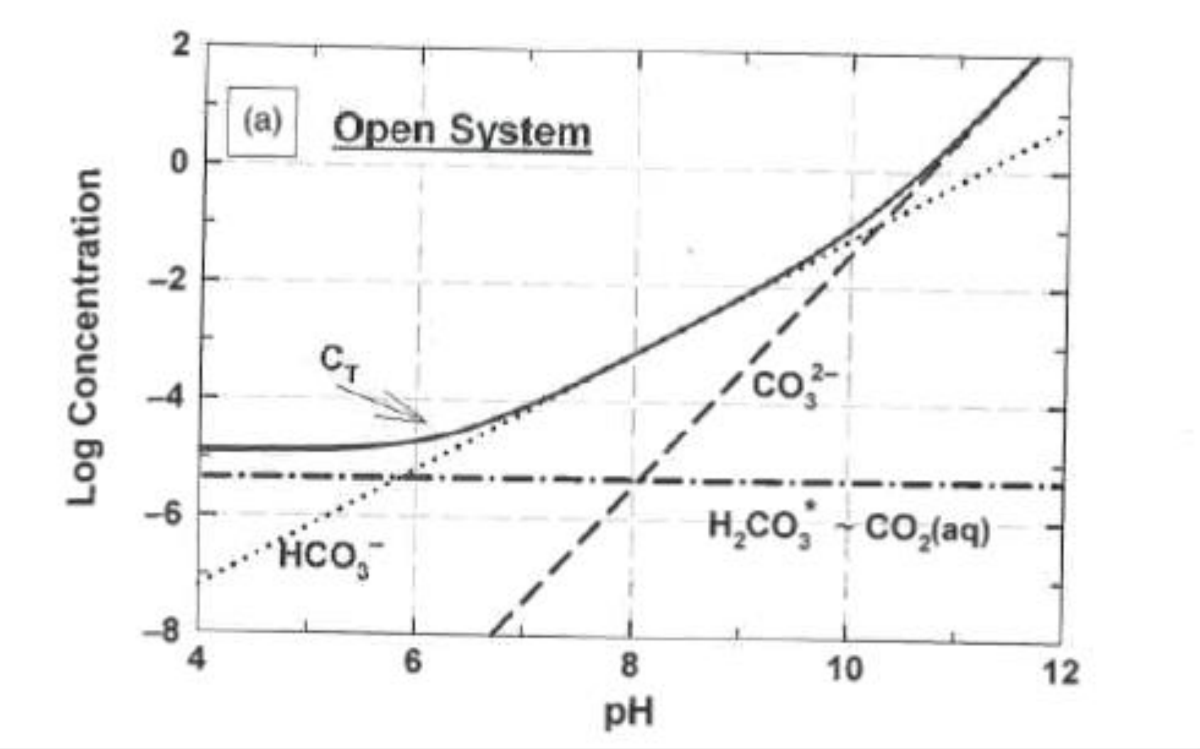
pH	H^+ M	α_0	α_1	α_2
2	1.0×10^{-2}	0.9999	5.01×10^{-5}	2.82×10^{-13}
3	1.0×10^{-3}	0.9995	5.01×10^{-4}	2.82×10^{-11}
4	1.0×10^{-4}	0.9950	4.99×10^{-3}	2.81×10^{-9}
4.5	3.16×10^{-5}	0.984	0.0156	2.78×10^{-8}
5	1.0×10^{-5}	0.952	0.0477	2.69×10^{-7}
5.5	3.16×10^{-6}	0.863	0.137	2.43×10^{-6}
6	1.0×10^{-6}	0.666	0.334	1.88×10^{-5}
6.3	5.01×10^{-7}	0.500	0.500	5.61×10^{-5}
7	1.0×10^{-7}	0.166	0.833	4.68×10^{-4}
7.5	3.16×10^{-8}	0.059	0.939	1.67×10^{-3}
8	1.0×10^{-8}	0.019	0.975	5.48×10^{-3}
8.3	5.01×10^{-9}	0.010	0.979	0.011
9	1.0×10^{-9}	1.88×10^{-3}	0.945	0.053
9.5	3.16×10^{-10}	5.30×10^{-4}	0.849	0.151
10	1.0×10^{-10}	1.30×10^{-4}	0.640	0.360
10.25	5.62×10^{-11}	5.50×10^{-5}	0.500	0.500
11	1.0×10^{-11}	3.0×10^{-6}	0.151	0.849
12	1.0×10^{-12}	3.4×10^{-8}	0.0175	0.983



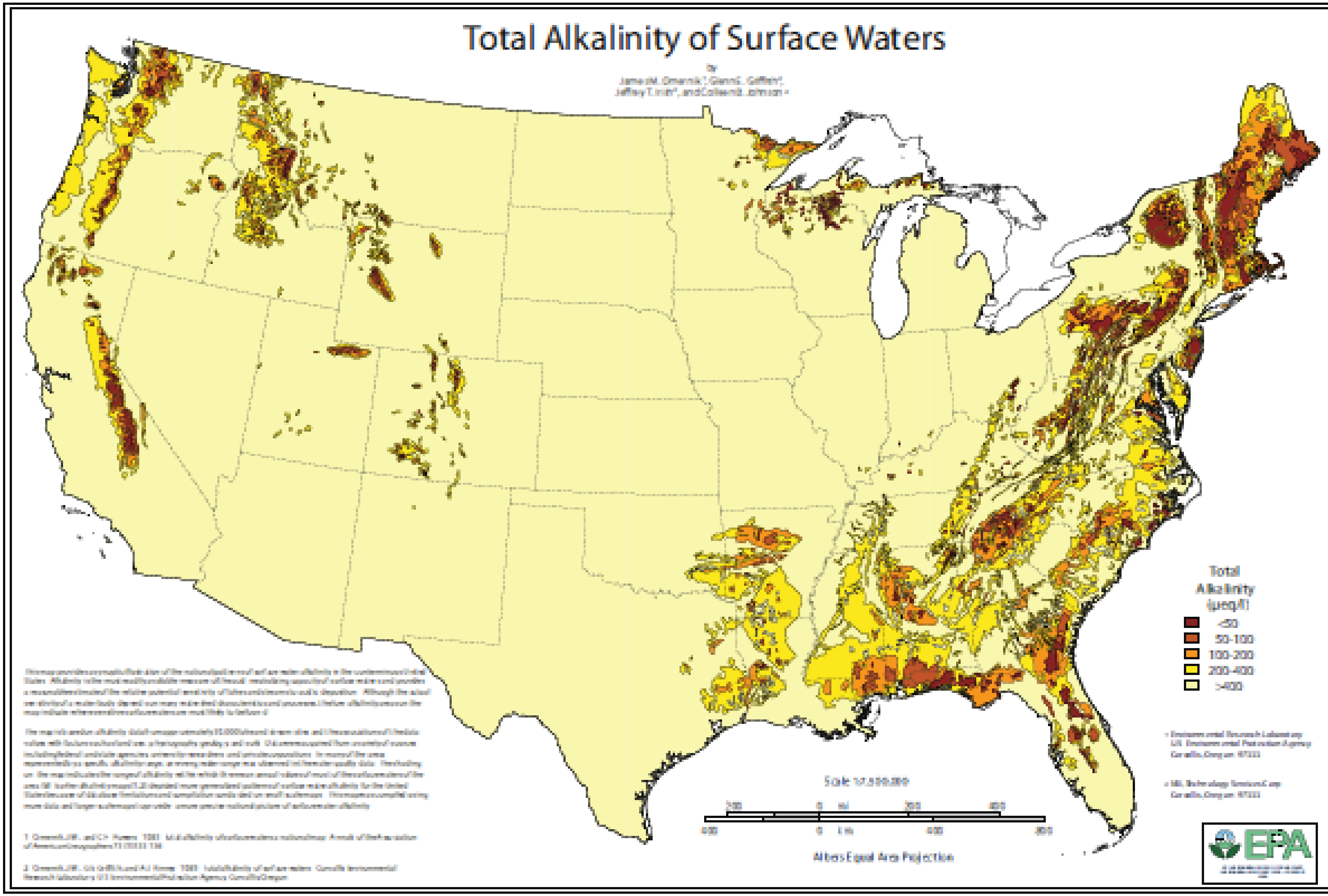
The Carbonate System, Open

- Carbon dioxide is a weak acid
- Dissolved readily in high pH solutions
- When open to the air, basic solutions will absorb CO_2

$C_{T,C}$ increases as pH increases to satisfy equilibrium



Alkalinity and Acidity

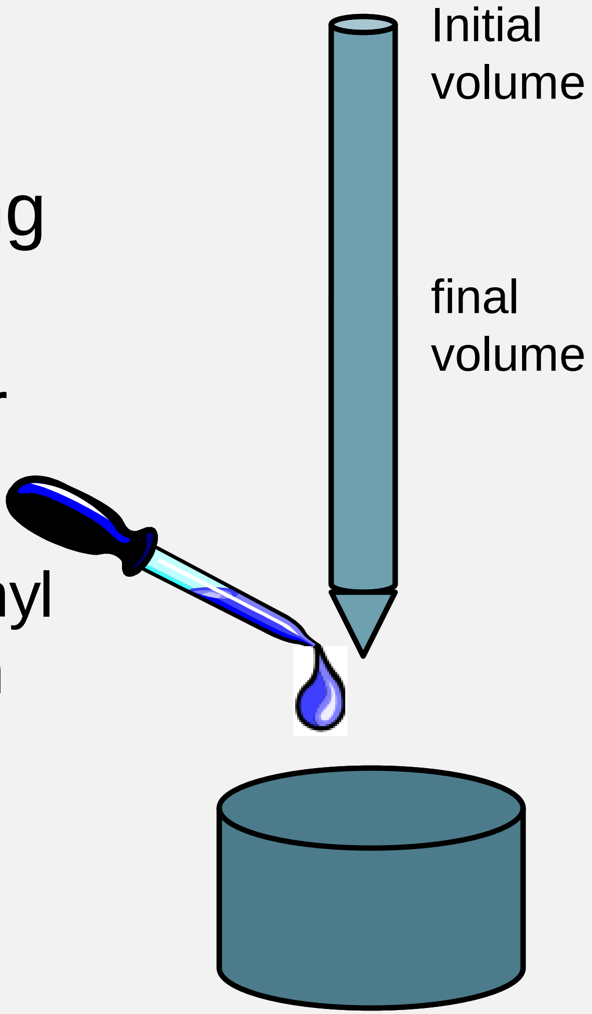


Alkalinity

- Alkalinity is the acid neutralization capacity of a water
  - Anything that can consume H^+ (acid) when added to water
  - Common Examples include:
    - Inorganic Carbon (HCO_3^-, CO_3^{-2})
    - Orthophosphate ($H_2PO_4^-$, HPO_4^{-2}, PO_4^{-3})
    - Also OH^- at basic pH
  - The *amount of strong acid* required to reduce pH to 4.5

Alkalinity

- Measuring Alkalinity
  - Titrate sample with a strong acid to pH 4.5
  - Measure with pH meter, or with indicator solution
    - Add bromcresol green-methyl red indicator solution to turn sample "pink"
    - Add strong acid (ie H_2SO_4) until color goes away
    - $vol_i - vol_f$ = meq Alkalinity



Alkalinity

- Usually see alkalinity in mg/L as $CaCO_3$
  - $(vol_{initial} – vol_{final})$ = meq Acid
  - 1 meq/L Acid = 50 mg/L as $CaCO_3$
- For example
  - Initial volume 0.02N H_2SO_4 = 52 mls
  - Final volume 0.02N H_2SO_4 = 47 mls

$$Alk = \frac{(52 - 45)mls\ x\ 0.02N\ x\ 50{,}000\frac{mgCaCO_3}{eq}}{100\ mls} = 70\frac{mg}{L}\ as\ CaCO_3$$

Alkalinity

- Inorganic Carbon and Alkalinity

  $Alk = [HCO_3^-] + 2[CO_3^{-2}]+[OH^-]-[H^+]$

  – Relating to C_T,

  $Alk = (\alpha_1 + 2\alpha_2)C_{T,C} + [OH^-]-[H^+]$

  – At near neutral pH (6 – 9)

  $Alk = [HCO_3^-] = \alpha_1 C_{T,C}$

$\alpha_0 \rightarrow H_2CO_3$
$\alpha_1 \rightarrow HCO_3^-$
$\alpha_2 \rightarrow CO_3^{2-}$

Acidity

- Acidity is the base neutralization capacity of a water
  - Anything that can donate H^+ (acid) or neutralize OH^- that is added to water
  - Common Examples include:
    - Inorganic Carbon (H_2CO_3, HCO_3^-)
    - Orthophosphate (H_3PO_4, $H_2PO_4^-$, HPO_4^{-2})
    - Ammonium Ion (NH_4^+)
    - Also H^+ at pH
  - The *amount of strong base* required to increase pH to 10.6

Acidity

- Inorganic Carbon and Acidity

  $$Acy = [H_2CO_3] + 2[HCO_3^-]+[H^+]-[OH^-]$$

  – Relating to C_T,

  $$Acy = (2\alpha_0 + \alpha_1)C_{T,C} + [H^+] - [OH^-]$$

  $\alpha_0 \rightarrow H_2CO_3$

  $\alpha_1 \rightarrow HCO_3^-$

  $\alpha_2 \rightarrow CO_3^{2-}$

  – Alkalinity and Acidity are related

  $$Alk + Acy = 2(C_{T,C})$$

Solubility & Precipitation

- Dissolving and forming of solids
- Example: table salt in water
  - Ability to dissolve is a function of:
    - Concentration
    - Temperature
    - pH (not here but sometimes)
    - Ionic strength
- Other examples
  - Sweep Floc formation
  - Sweet Tea
  - Lime Softening

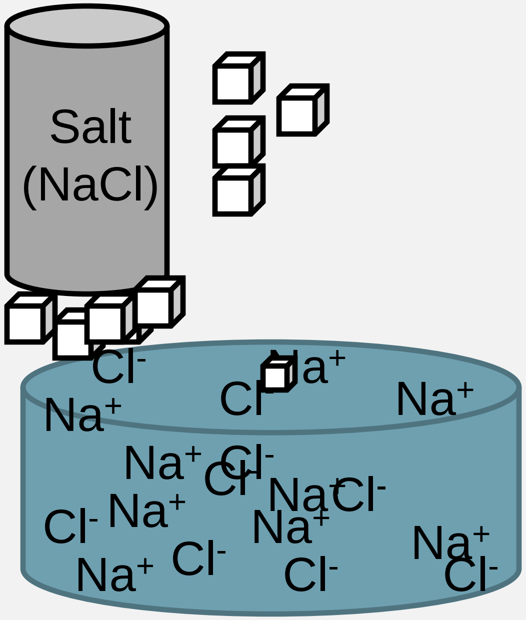
Disolution

Undersaturation

Saturation

Supersaturation

Precipitation

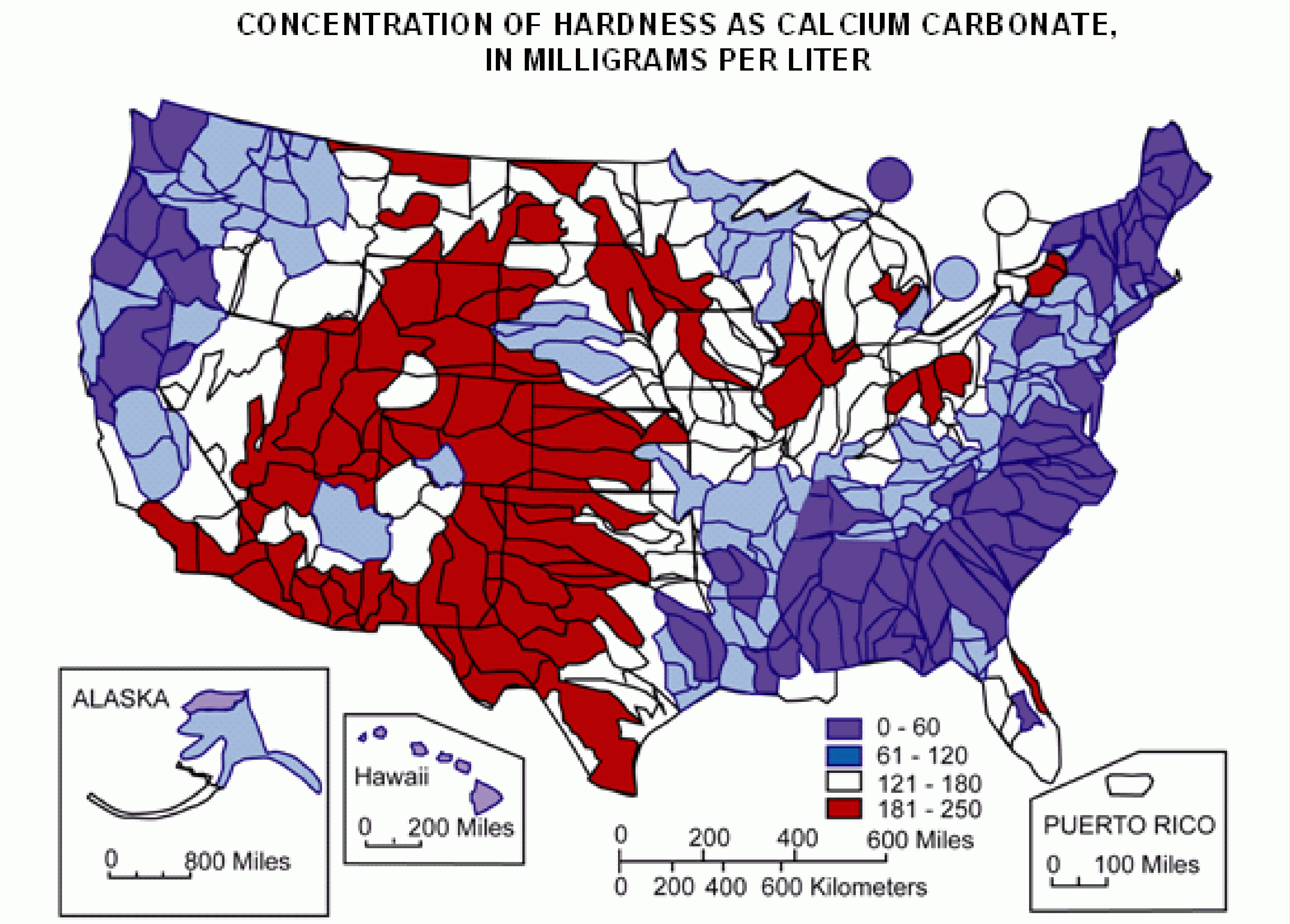


Solubility & Precipitation

- Solubility Equilibria
  - $A_xB_y\ (s) \leftrightarrow xA^{y+} + yB^{x-}$
  - $K_{sp} = [A^{y+}]^x\, [B^{x-}]^y$
- If $[A^{y+}]^x\, [B^{x-}]^y > K_{sp}$, precipitation can occur
- Some K_{sp} values:

Solid	Formula	K_{sp}
Aluminum hydroxide	$Al(OH)_3$	$2x10^{-32}$
Calcium carbonate (calcite)	$Ca(CO_3)_2$	$3.31x10^{-9}$
Calcium hydroxide	$Ca(OH)_2$	$5.02x10^{-6}$
Ferrous carbonate	$FeCO_3$	$3.13x10^{-11}$
Ferrous hydroxide	$Fe(OH)_2$	$4.87x10^{-17}$
Ferric hydroxide	$Fe(OH)_3$	$2.79x10^{-39}$
Lead(II) hydroxide	$Pb(OH)_2$	$1.43x10^{-20}$
Lead (VI) hydroxide	$Pb(OH)_4$	$3.2x10^{-66}$
Magnesium hydroxide (amorphous)	$Mg(OH)_2$	$3.91x10^{-11}$

Water Hardness



Water Hardness

- Presence of multivalent cations in water (expressed as mg/L as $CaCO_3$)
  - Ca^{2+}
  - Mg^{2+}
- Aesthetic water issues with hardness
  - Scaling of boilers, water heaters, washing machines, coffee pots, and other hot water appliances
  - Conservation of soaps and detergents
  - Taste
    - (Durand and Dietrich, 2009)

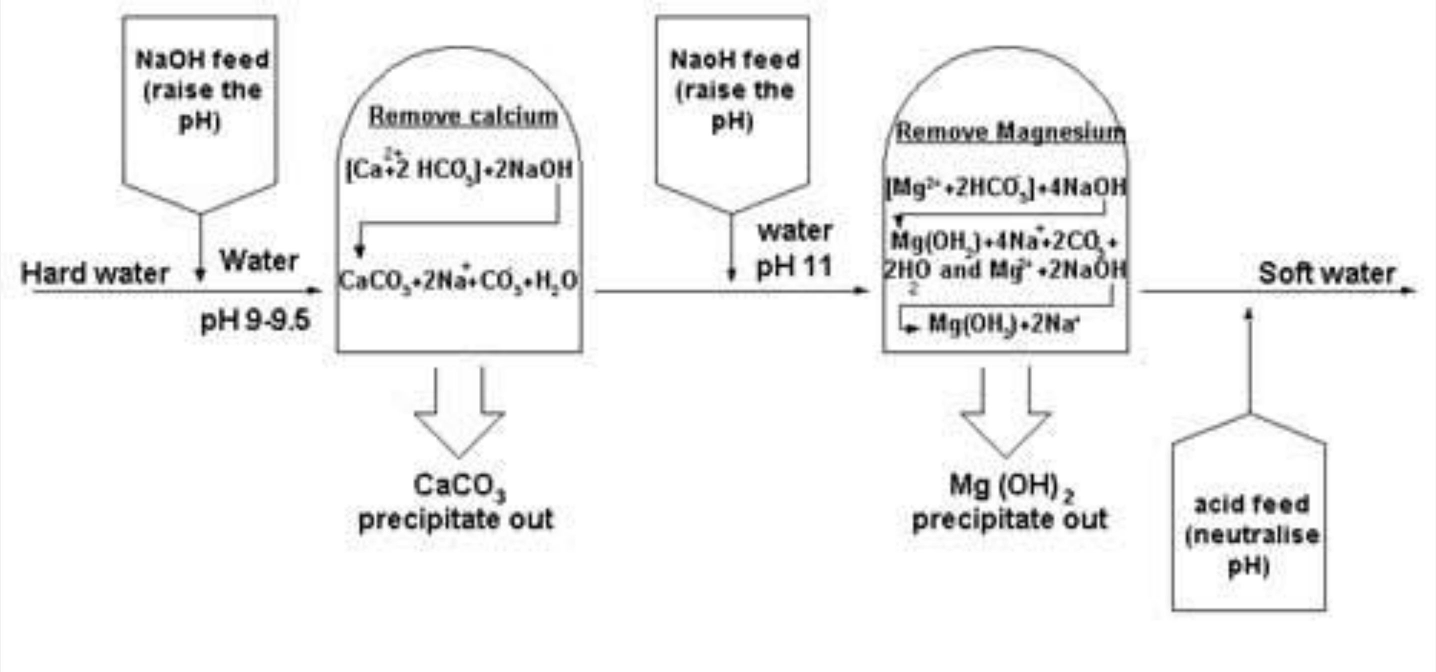
Hardness (mg/L as $CaCO_3$)	Degree of Hardness
0 – 75	Soft
75 – 150	Moderately hard
150 – 300	Hard
> 300	Very hard

Water Hardness

- Carbonate vs. non-carbonate hardness
  - Operational definition tied to alkalinity
  - Addition of lime will change bicarbonate to carbonate, which will precipitate Ca^{2+} or Mg^{2+}
    - Carbonate Hardness
  - When alkalinity exhausted, Ca^{2+} and Mg^{2+} cannot precipitate with lime addition.
    - Noncarbonate Hardness
    - The addition of sodium carbonate or carbon dioxide can remove NCH

Water Hardness

- Treatment involves precipitation, ion exchange, or membrane softening/desalting
- Precipitative Softening



Water softening by the chemical precipitation process

Water Hardness Example

- Question 1: Estimate residual Mg^{2+} in lime-treated water assuming it is limited by $Mg(OH)_2(s)$ solubility, and the pH is 11.0
- Question 2: How much hardness remains in solution (as $CaCO_3$)?

Water Hardness Example

- Question 1 Solution:
- $K_{sp} = [Mg^{2+}][OH^{-}]^{2} = 3.91x10^{-11}$
- pH = 11 → pOH = 14-11 = 3
- $-log[OH^{-}]=3$ → $[OH^{-}]=10^{-3}$
- $[Mg^{2+}][1x10^{-3}]^{2} = 3.91x10^{-11}$
- $[Mg^{2+}] = 3.91x10^{-11} / 1x10^{-6} = 3.91x10^{-5}$ M
- $3.91x10^{-5}$ M = 0.95 mg/L Mg^{2+}

Water Hardness Example

- Question 2 solution:
- Only hardness that remains is 0.95 mg/L Mg^{2+}

$$\frac{0.95 \text{ mg } Mg^{2+}}{L} \times \frac{1 \text{ mmol } Mg^{2+}}{24.3 \text{ mmol } Mg^{2+}} \times \frac{2 \text{ meq Hardness as } CaCO_3}{2\text{meq } Mg^{2+}} \times \frac{100 \text{ mg Hard}}{\text{meq Hard}}$$

- 3.91 mg/L Hardness as $CaCO_3$

SECTION 2: LABORATORY PRACTICES AND CALCULATIONS

Dimensional Analysis
Making Dilutions / Serial Dilutions
Making Standards from Stock Solutions
Titrations

Dimensional Analysis

- The technique of converting between units
  - Conversion factor – an equation (or fraction) to relate two units
  - Easy Examples:
    - How many milligrams are in a gram?
    - How many centimeters are in a meter?

Dimensional Analysis

- Step-by-step instructions for converting units
  1. On the left, write the unit you are looking for (box it off)
  2. Next to that, write the value and unit you are given
  3. Choose a conversion factor that relates those 2 units
  4. Place the value with the unit you *have* on the bottom and the value with the unit you are *looking for* on top
  5. Cancel units
  6. Multiply by the numerator, divide by the denominator
  7. Write your answer on the right side, circle it

Dimensional Analysis

- A more complicated example
  - How many milliliters are in a 17 gallons?

$\boxed{\text{milliliters}}$ 17 gallons

- Conversion Factor: 1 gallon = 3785 milliliters

17 gallons	3785 milliliters	64,345 milliliters
	1 gallon	

A fun (and expensive) example

- The grain is a strange British unit that is still in use today.
  - It derives from the average mass of a barley seed, with 1 gram consisting of 15.43 grains.
  - The carat (as in diamonds!) is the average mass of a carob seed and equal to 3 grain.
- Question: What is the mass in grams of a 1.0 carat diamond?
  - How many Carbon atoms are in a 1.0 carat diamond ring?

Solution

- How many grams in a 1 carat diamond

$\boxed{\text{grams}}$ 1 carat

- Conversion factor: carat → grain → grams
  - 1 carat = 3 grains
  - 1 gram = 15.43 grains

1 carat	3 grains	1 gram	0.19 grams
	1 carat	15.43 grain	

- 1 mol = 12 grams C = 6.023×10^{23} C atoms
  - 1 carat = 9.5×10^{21} C atoms

Making Dilutions / Serial Dilutions

- A few terms and conversions of note
  - milligram = mg = 1/1000 of a g or 10^{-3} g
  - gram = g
  - kilogram = kg = 1000 g or 10^{3} g
  - mole = 6.023×10^{23} molecules
  - molarity = moles per liter
    - molar = M = term used to discuss molarity of solutions
  - millimole = 1/1000 of a mole
    - millimolar = mM = term used to discuss molarity in thousandths of a mole
  - w/v = weight (of a solute) per final solution volume
  - v/v = volume (of reagent) per final solution volume

Simple Dilution

- Aka – dilution factor method
  - A unit volume of a liquid material of interest is combined with an appropriate volume of solvent liquid to achieve the desired concentration
  - Example
    - 1:5 dilution = 1 unit of dilutent + 4 units of solvent

Simple dilution examples

- How do you make a 1:300 dilution of a bacillus spore sample?
  - 1 ml of sample + 299 mls solvent
- How do you dilute a 20X solution to the appropriate concentration before use?
  - Needs to be diluted 1:20
  - 1ml of 20x solution + 19ml of solvent

Mixing parts or volumes

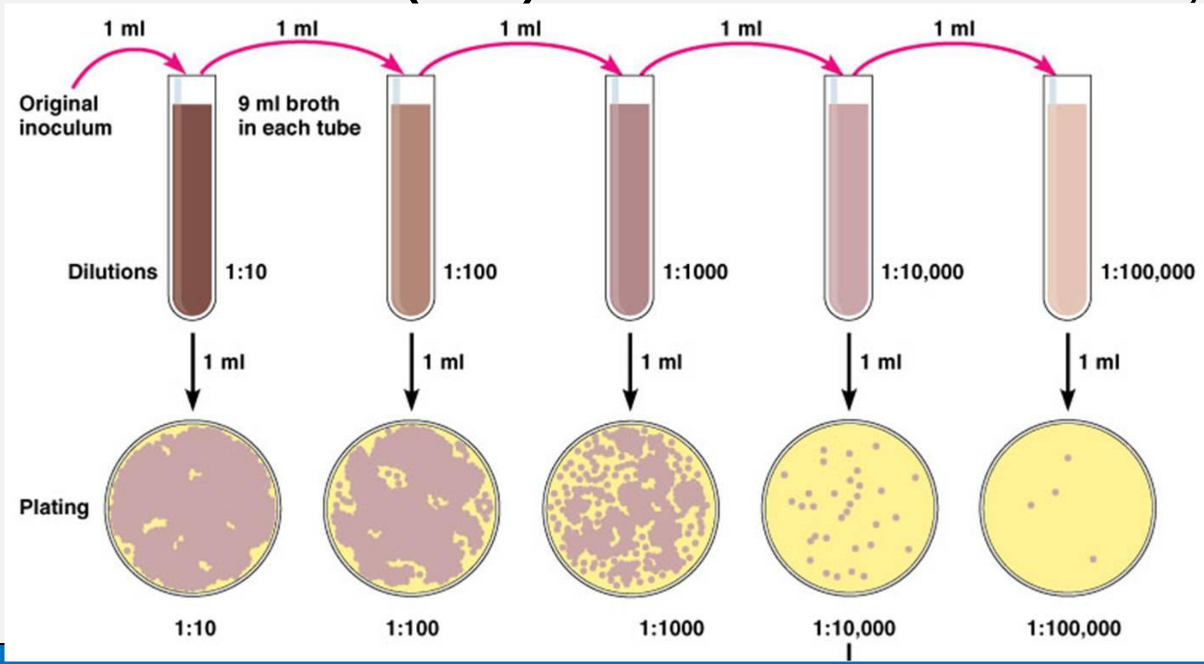
- Different from simple dilution
- Example
  - Instructions state to mix 1 part acid with 3 parts water. What do you do and how is this different from simple dilution?
  - Solution
    - Literally mix 1 unit acid (eg 1 L) with 3 units water (eg 3 L)
    - Creates 1:4 dilution
- Remember: Always add acid to water!!!

Why not to add water to acid

“Safety Third, is what I always say”

Serial Dilution

- A series of simple dilutions with amplifies the dilution factor quickly beginning with a small initial quantity of material
  - Good for DNA, RNA, enzyme work
- Final dilution factor (DF) = DF1 x DF2 x DF3, *etc.*



Making Standards from Stock Solutions

- Often need to make fixed volumes of solutions of known concentrations from stock solutions
  - Expensive, limited materials
  - Waste concerns
  - Good lab practice
  - Convenience
- Key equation:
  - $V_1C_1 = V_2C_2$

Percent Solutions

- Aka, parts per hundred
  - With a dry chemical, it is mixed as *dry mass per volume*, where g/100ml = % concentration
  - With liquid reagents, % concentration based upon *volume per volume* = ml/100ml
- Dry chemical example:
  - 3% w/v NaCl
  - Dissolve 3.0 g NaCl in 100ml water
- Liquid chemical example:
  - 70% v/v ethanol
  - Mix 70ml of 100% ethanol with 30ml water
- Need to keep in mind specific gravity for “non-water like” liquids

Example

- Dilute on-site alum for jar tests

Step 1: Calculating Chemical Concentrations

<u>On-site Alum:</u>

48.5% strength, specific gravity (S.G.) = 1.33

Stock Concentration (mg/mL) =

Percent Strength x S.G. x 10

Alum stock = 48.5 x 1.33 x 10

= 645 mg/mL

= 645,000 mg/L

Making Secondary Stocks

Alum Primary Stock = 645 mg/mL

Need a secondary stock of 20 mg/mL (500 mL)

X * 645 = 20 * 500

X = 15.5 mL

Add 15.5 mL of primary stock to a 500 mL volumetric flask

Secondary alum stocks are usually good for 2 hours (will hydrolyze after that)

Using Secondary Stock for Jar Tests

Alum Secondary Stock = 20 mg/mL

Each Square Beaker = 2 Liters

<u>1 mL of secondary stock to a beaker</u>:

= 20 mg/mL * 1mL / 2 L

= Alum Dose of 10 mg/L

Side Note: Calculating Chemical Feed Rates

Alum Primary Stock = 645 mg/mL = 645,000 mg/L

Pilot Plant Flow Rate = 15 gpm

Required Alum Dose = 40 mg/L

$$\text{Alum Pump Setting} = \frac{15\ \text{gal/min} \times 3.785\ \text{L/gal} \times 40\ \text{mg/L}}{645{,}000\ \text{mg/L}}$$

$$= 0.0035\ \text{L/min}$$

$$= 3.5\ \text{mL/min}$$

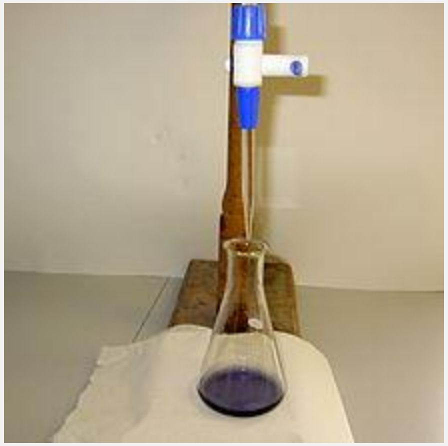
Polymer Stocks

- Polymers are usually very viscous (liquids)
- Easier to weigh them rather than dispense a volume

- Say, need to make a polymer stock of 0.2 mg/mL (500 mL volume):
  - Amount of polymer required = 0.2 x 500 = 100 mg
  - Polymer doses are usually as product
  - Weigh 100 mg of polymer, transfer to a 500 mL volumetric flask, and make the volume to 500 mL

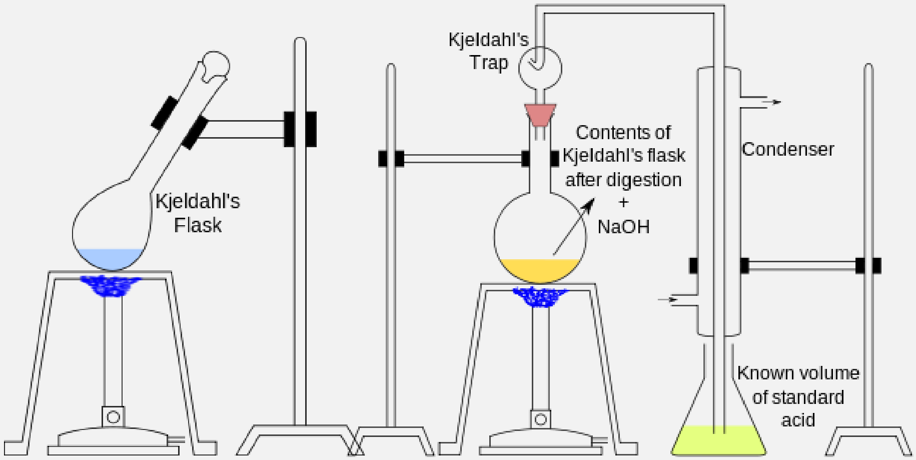
Titrations

- Common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of an identified analyte.
- Types of Titrations:
  - Acid/Base
  - Redox
  - Complexation
  - Zeta Potential
  - Assay



Titrations

- Several Examples
  - **Alkalinity**
  - **Acid Value**
  - Kjeldahl Nitrogen
  - Dissolved Oxygen
  - Benedict's reagent
  - Bromine/Iodine Number



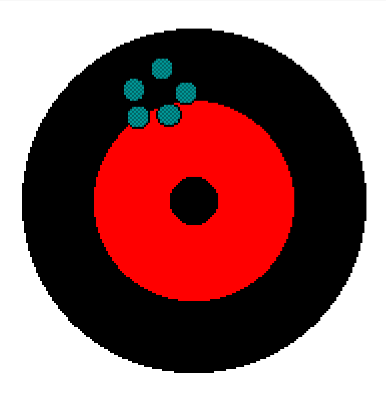
- See Alkalinity discussion in Section 1 for example calculations

SECTION 3: STATISTICS

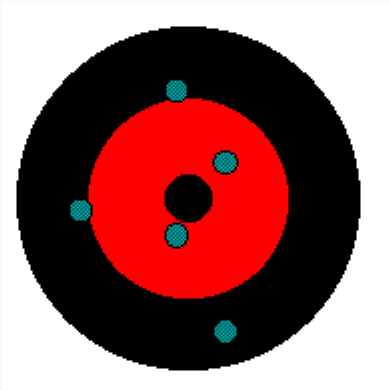
Precision and Accuracy
Significant Figures
Basic Statistics
Calculating RPD/RSD/% recovery
Understanding the 95% Confidence Interval
The theory behind correlation coefficients

Precision and Accuracy

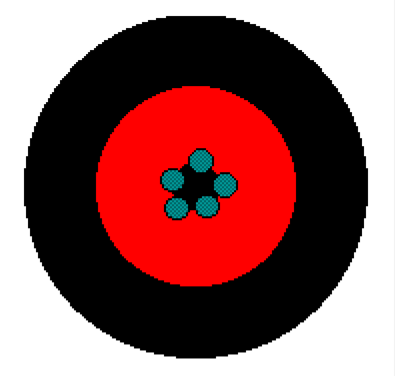
- Precise: After taking a lot of measurements, you notice that they are all very close to each other.
- Accurate: After taking a lot of measurements, you find they agree with the true value
- Dart Board Example:



Precise, not accurate



Neither Precise nor accurate



Precise and accurate

Significant Figures

- Only report numbers with the correct degree of precision
- Significant Figures are the digits in a number which are known precisely, plus one estimated digit

What is the temperature, to the correct significant digit?

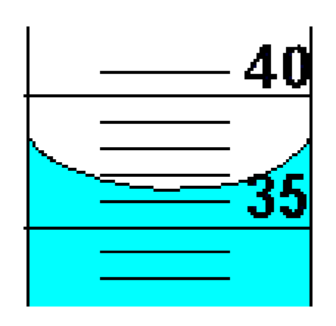
18.5^{o}C

Counting Significant Figures

- Only a few simple rules
  - All non-zero numbers *are* significant
    - Eg (sig figs in parenthesis): 251 (3), 13.49 (4), 8765.1 (5)
  - Zeros *between* significant digits are significant
    - Eg: 305 (3), 42003 (5), 70201 (5)
  - If there is no decimal point, than *trailing* zeros are *not* significant
    - Eg: 470 (2), 10 (1), 6,000 (1)
  - If a number is less than one, then the fist significant figure is the first *non-zero* digit after the decimal point
    - Eg: 0.009 (1), 0.156 (3), 0.01060 (4)

Measuring with Significant Figures

- Using the correct number of significant digits shows how precise your measurement was
- Result should have all of the digits which you're sure of an then one estimated digit
- What is the volume in the graduated cylinder below:



36.5^{o}C

Calculating with Significant Figures

- When performing calculations involving lab results, must be aware of significant figures:
- Follow these rules:
  - When adding or subtracting, the number of digits to the right of the decimal point in the answer is equal to the number of digits to the right of the decimal point in the number with the least such digits
  - When multiplying or dividing, the answer should have the same number of sig figs, as the number with the fewest sig figs.

Calculating with Significant Figures

- Give the answers to the following, with the correct number of significant figures:

$$\begin{array}{r} 36.45 \\ +\ 1.467 \\ \hline \end{array}$$

37.91

$45 \times 21.31111 =$ 960

$9{,}081.3 \div 3 =$ 3,000

$$\begin{array}{r} 1.7 \\ -\ 0.1357 \\ \hline \end{array}$$

1.6

Basic Statistics

- Populations, Parameters, Samples, Variables and Statistics

Population

the aggregate of all arbitrarily defined sample units

- Parameters

constants that describe the population as a whole

Samples

an aggregation of sample units

- Variables

a characteristic that may vary from one sample to the next

- Statistic

parameter of a sample (sample distribution)

Basic Stats

- What’s a sample?
  - Any subset (or collection) of units from a population of units
    - May be the units themselves (e.g., A handful of Reeses pieces from a large jar of pieces)
  - or more often
    - A measure of the units (e.g., a list of heights from 10 trees occurring in a stand of 1200 trees).
- Samples that are selected at random, or similar to random, may lend themselves to statistical analysis

Basic Stats

- Why sample?
  - Measuring all units (trees, recreationists, birds, etc.) is impractical, if not impossible.
  - Sampling just a few units saves money.
  - Sampling just a few units saves time.
  - Some measurements are destructive:
    - cutting down trees to inspect ring patterns or stem analysis
    - capturing wildlife to examine their morphology, etc.
- **Sampling makes statistical methods attractive and powerful.**

Basic Stats – Frequency Distribution

- Normal Distribution
  - Most commonly used by scientists and lab practitioners
  - Means of large samples are expected to have a distribution that approaches normality

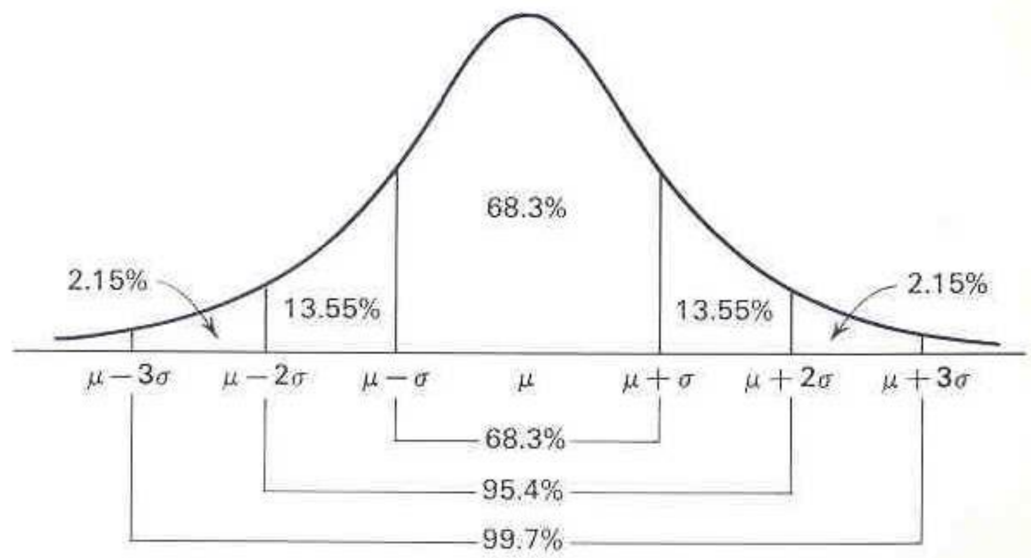


Figure 8.11 Distribution of elements in a $N(\mu, \sigma^2)$ population.

Basic Stats – Frequency Distribution

Useful Statistics:

Mean,
Standard Error
Coefficient of Variance
Standard Deviation
Confidence

Compare means

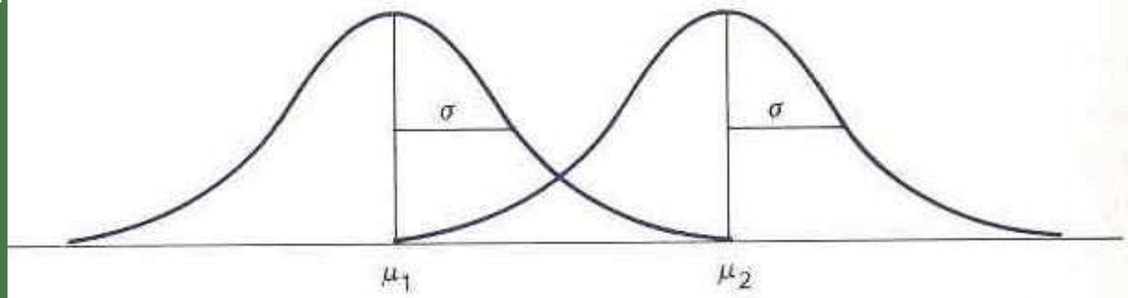


Figure 8.9 Normals with different means, same variance.

Compare dispersion

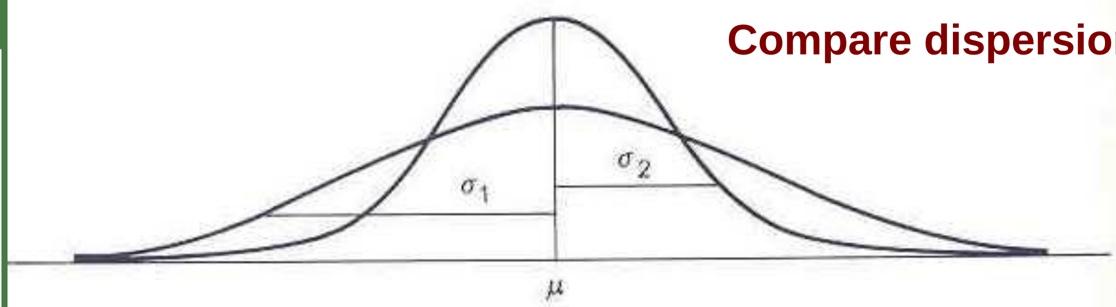


Figure 8.10 Normals with different variances, same mean.

Basic Stats – Frequency Distribution

- There are other distributions useful in statistics, including:
  - Chi Square
  - F
  - Student’s t-distribution
  - Binomial
  - Negative Binomial
  - Gamma
- Sometimes you may see the Normal referred to as Gaussian.
- We will not consider other distributions in this workshop

Basic Statistics

- Terms

$$n = total\ \ number\ \ of\ \ samples$$

$$x = sample$$

$$\bar{x}\ \ (or\ \ \mu) = sample\ \ average$$

$$s^2 = variance$$

$$s\ \ (or\ \ \sigma) = standard\ deviation$$

$$CV\ \ = coefficient\ \ of\ variation$$

$$s_{\bar{x}}\ or\ SE = standard\ \ error\ \ of\ \ the\ \ mean$$

Basic Stats - Computations

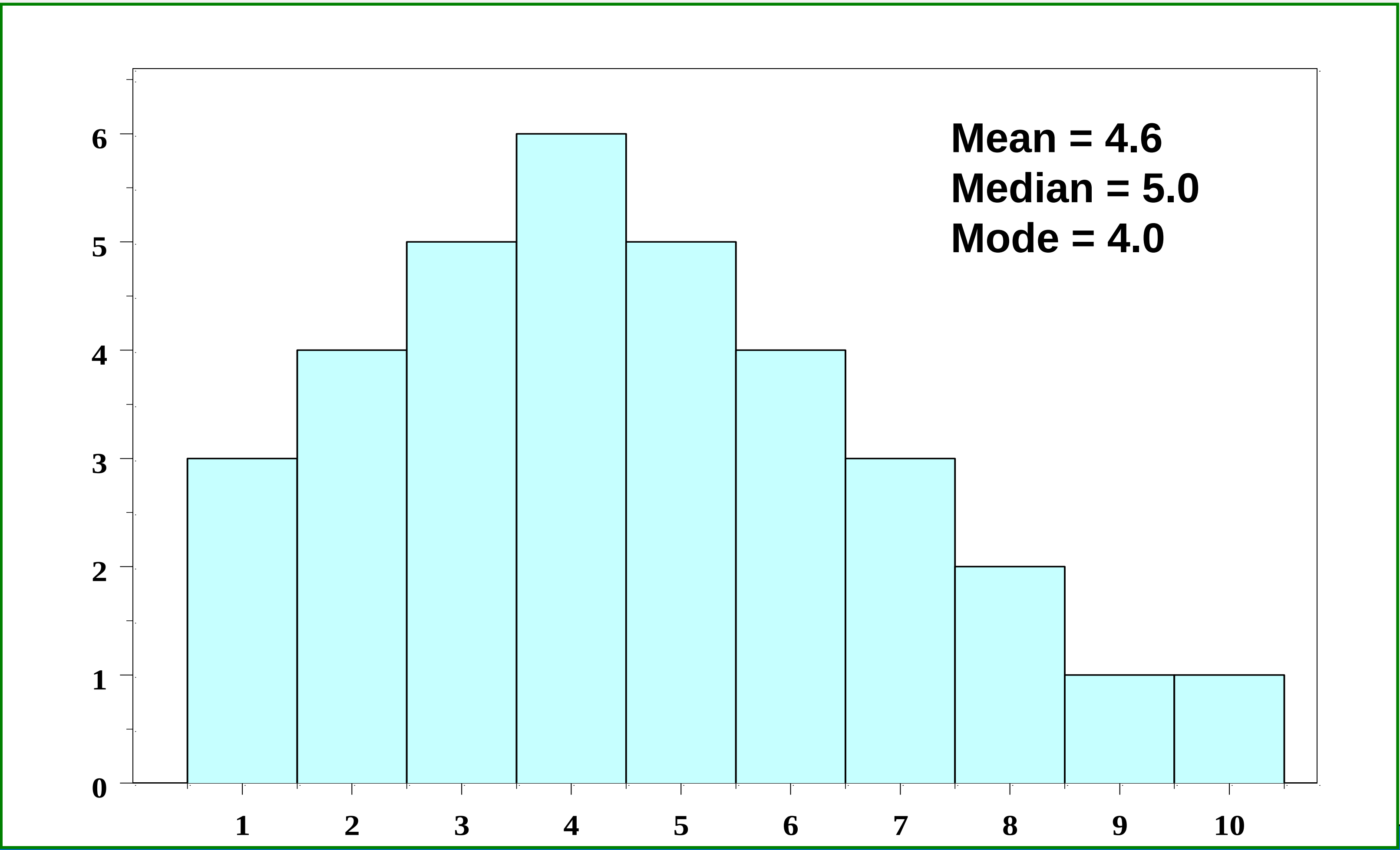
- Mean (the average)
- Median (the middle value)
- Mode (the most frequently appearing value)

$$\bar{x} = \left(\frac{\sum x}{n} \right)$$

Mean

Basic Stats – Computations

- Mean, Median, and Mode



Basic Stats – Computations

- Range
  - easy to compute
  - fails to take into account how the data are distributed

$$\boldsymbol{Range = x_{(max)} - x_{(min)}}$$

Basic Stats – Computations

- Measures of Dispersion

Variance

- A measure of dispersion among individual observations about their average value
- Computed before the standard deviation

$$s^2 = \left(\frac{\sum x^2 - \left(\left(\sum x \right)^2 / n \right)}{n - 1} \right)$$

Standard deviation

- Another measure of dispersion
- 68% of observations should be within ± 1 standard deviation of the mean
- 95% will be within 1.96 standard deviations

$$s = \sqrt{\frac{\sum x^2 - \left(\left(\sum x \right)^2 / n \right)}{n - 1}}$$

Basic Stats – Computations

- Alternate formulas for variance and standard deviation

Variance

$$s^2 = \left(\frac{\sum (\bar{x} - x)^2}{n-1} \right)$$

Standard deviation

$$s = \sqrt{\left(\frac{\sum (\bar{x} - x)^2}{n-1} \right)}$$

Basic Stats – Computations

- Coefficient of variation
  - Permits a comparison of relative variability about means of different sizes, and data collected from different populations
  - A relative measure, expressed as a percentage the ratio of the standard deviation to the mean of a sample

$$CV = \left(\frac{s}{\bar{x}}\right) 100\%$$

Basic Stats – Computations

- Standard error of the mean
  - A measure of variation of among sample means (place different means on a common reference) calculated from the same population
  - Used to help produce confidence limits, or to determine required sample sizes for a sampling effort
  - Assumes random sampling from an infinite population

$$SE = \left(\sqrt{\frac{s^2}{n}}\right)$$

$$SE = \left(\frac{s}{\sqrt{n}}\right)$$

Basic Stats – Computations

- Standard error of the mean in percent
  - A measure of variation of among sample means (place different means on a common reference)

$$SE\% = \left(\frac{CV}{\sqrt{n}} \right)$$

Basic Stats - Example

Sample of student ages:
19, 21, 21, 24, 30, 20

Sample size:
$n = 6$

$n-1 = 5$
$\sqrt{n} = 2.45$

1 Mean age:
mean = 19 + 21 + 21 + 24 + 30 + 20 / 6
mean = 22.5

2 Variance of the mean:
$s^2 = 81.5 / 5$
$s^2 = 16.3$

3 Standard Deviation of the mean:
SD = $\sqrt{16.3}$
SD = 4.04

4 Standard Error of the mean:
SE = 4.04 / 2.45
SE = 1.65

5 Coefficient of Variation:
CV = 4.04 / 22.5
SE = 0.18 or 18%

x	n
19	6
21	6
21	6
24	6
30	6
20	6

Calculating RPD/RSD/% recovery

- RPD = Relative Percent Difference
- RSD = Relative Standard Deviation
- % Recovery = Percent of known *spiked* analyte concentration measured upon analysis

RPD

- The RPD is used with two measurements exist.
  - Expresses the precision of duplicates

$$RPD = \frac{|X_1 - X_2|}{\bar{X}} * 100$$

    - x1 = measurement #1
    - x2 = measurement #2
    - $\bar{x}$ = mean of measurements

RSD

- The RSD is used when there are at least three measurements
  - Expresses the precision of measurements

$$RSD = \frac{s}{\bar{x}} * 100$$

- S = standard deviation
- $\bar{x}$ = mean of measurements

% Recovery

- Measures the accuracy of a technique

$$\%R = \frac{(SSR - SR)}{SA} * 100$$

  - SSR = measured value of spiked sample
  - SR = measured value of sample
  - SA = known value of spike added
- If there is no sample contribution to the measured values, SR = 0

$$\%R = \frac{SSR}{SA} * 100$$

Understanding 95% Confidence Intervals

- If the population is normally distributed, the Central Limit Theorem indicates that 95% of all *sample means* are within 2 Standard Errors (SE) of the *population mean*
- Assuming a Normal Distribution, 95% confidence intervals can be calculated using the sample mean and sample standard deviation

Calculating 95% Confidence Intervals

- Step 1: Calculate Standard Error (SE)
  - S = sample standard deviation
  - N = sample size

$$SE = \frac{s}{\sqrt{N-1}}$$

- Step 2:

$$\text{CI } 95\% = \bar{X} \pm t * (SE)$$

  - X = sample mean
  - t = 1.96 for normal distribution, 95% CI

95% CI Example

Sample mean = 60.5

Sample standard deviation = 24

Sample size N = 101

Calculate the 95% CI

$$SE = \frac{s}{\sqrt{N-1}} = \frac{24}{\sqrt{101-1}} = \frac{24}{\sqrt{100}} = \frac{24}{10} = 2.4$$

95% Confidence Interval:

60.5 $\pm$ (1.96 * 2.4) = 60.5 $\pm$ 4.7

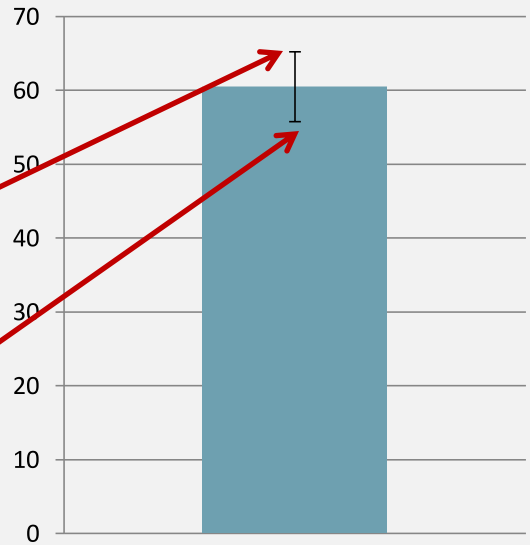
t

The upper bound of the interval is:

60.5 + (1.96 * 2.4) = 65.2

The lower bound of the interval is:

60.5 – (1.96 * 2.4) = 55.8



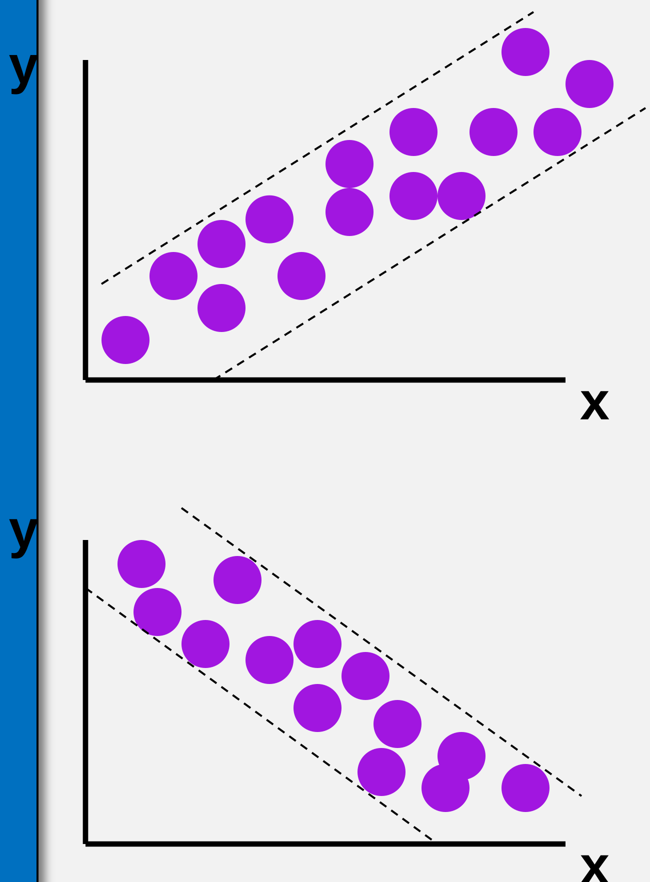
Scatter Plots and Correlation

- A scatter plot (or scatter diagram) is used to show the relationship between two variables
- Correlation analysis is used to measure strength of the association (linear relationship) between two variables
  - Only concerned with strength of the relationship
  - No causal effect is implied

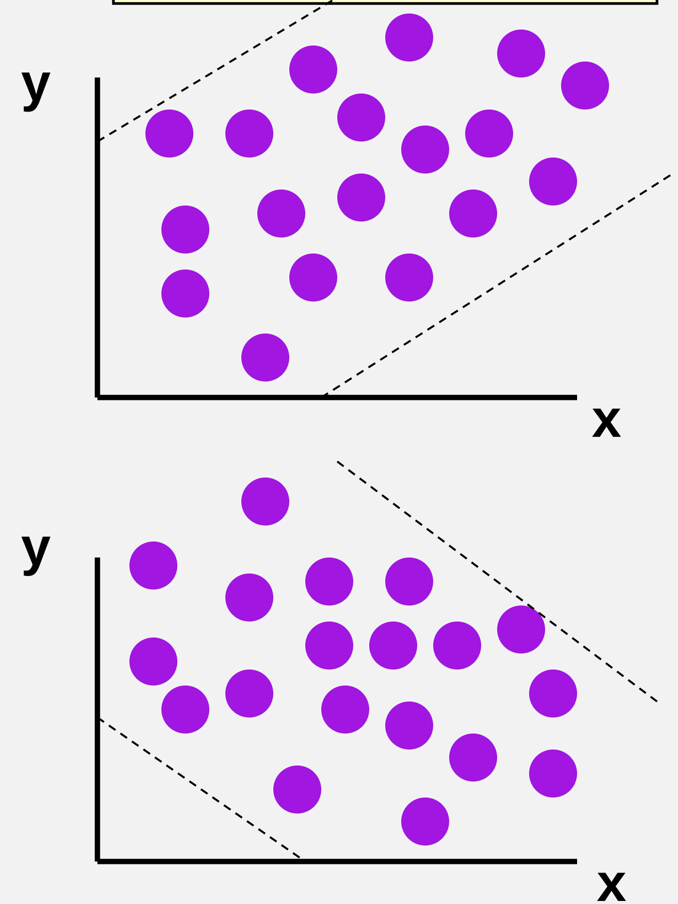
Scatter Plot Examples

(continued)

Strong relationships



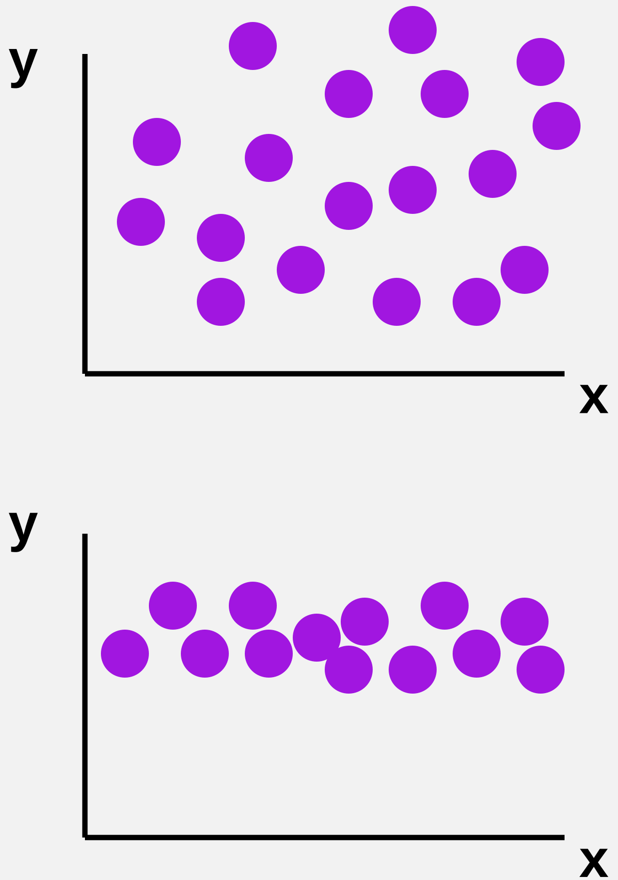
Weak relationships



Scatter Plot Examples

(continued)

No relationship



Correlation Coefficient

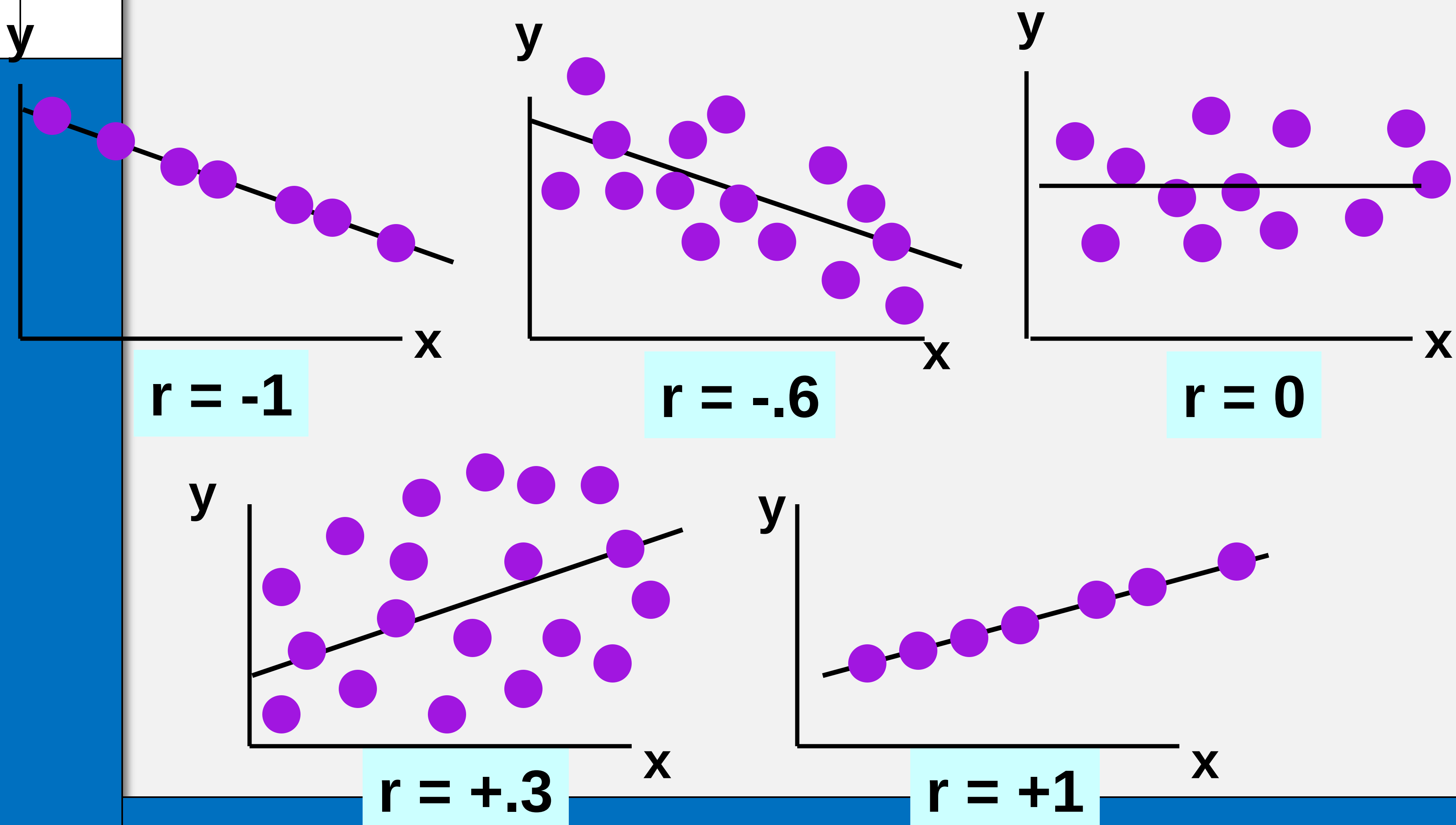
(continued)

- The population correlation coefficient ρ (rho) measures the strength of the association between the variables
- The sample correlation coefficient r is an estimate of ρ and is used to measure the strength of the linear relationship in the sample observations

Features of ρ and r

- Unit free
- Range between -1 and 1
- The closer to -1, the stronger the negative linear relationship
- The closer to 1, the stronger the positive linear relationship
- The closer to 0, the weaker the linear relationship

Examples of Approximate r Values



Calculating the Correlation Coefficient

Sample correlation coefficient:

$$r = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{[\sum (x - \bar{x})^2][\sum (y - \bar{y})^2]}}$$

or the algebraic equivalent:

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{[n(\sum x^2) - (\sum x)^2][n(\sum y^2) - (\sum y)^2]}}$$

where:

r = Sample correlation coefficient
n = Sample size
x = Value of the independent variable
y = Value of the dependent variable

Coefficient of Determination, R^2

- The coefficient of determination is the portion of the total variation in the dependent variable that is explained by variation in the independent variable

- The coefficient of determination is also called R-squared and is denoted as R^2

$$R^2 = \frac{SSR}{SST}$$ where $$0 \leq R^2 \leq 1$$

Coefficient of Determination, R^2

(continued)

Coefficient of determination

$$R^2 = \frac{SSR}{SST} = \frac{\text{sum of squares explained by regression}}{\text{total sum of squares}}$$

Note: In the single independent variable case, the coefficient of determination is

$$R^2 = r^2$$

where:

R^2 = Coefficient of determination

r = Simple correlation coefficient

- Total variation is made up of two parts:

$$SST = SSE + SSR$$

Total sum of Squares

Sum of Squares Error

Sum of Squares Regression

$$SST = \sum (y - \bar{y})^2$$

$$SSE = \sum (y - \hat{y})^2$$

$$SSR = \sum (\hat{y} - \bar{y})^2$$

where:

$\bar{y}$ = Average value of the dependent variable

y = Observed values of the dependent variable

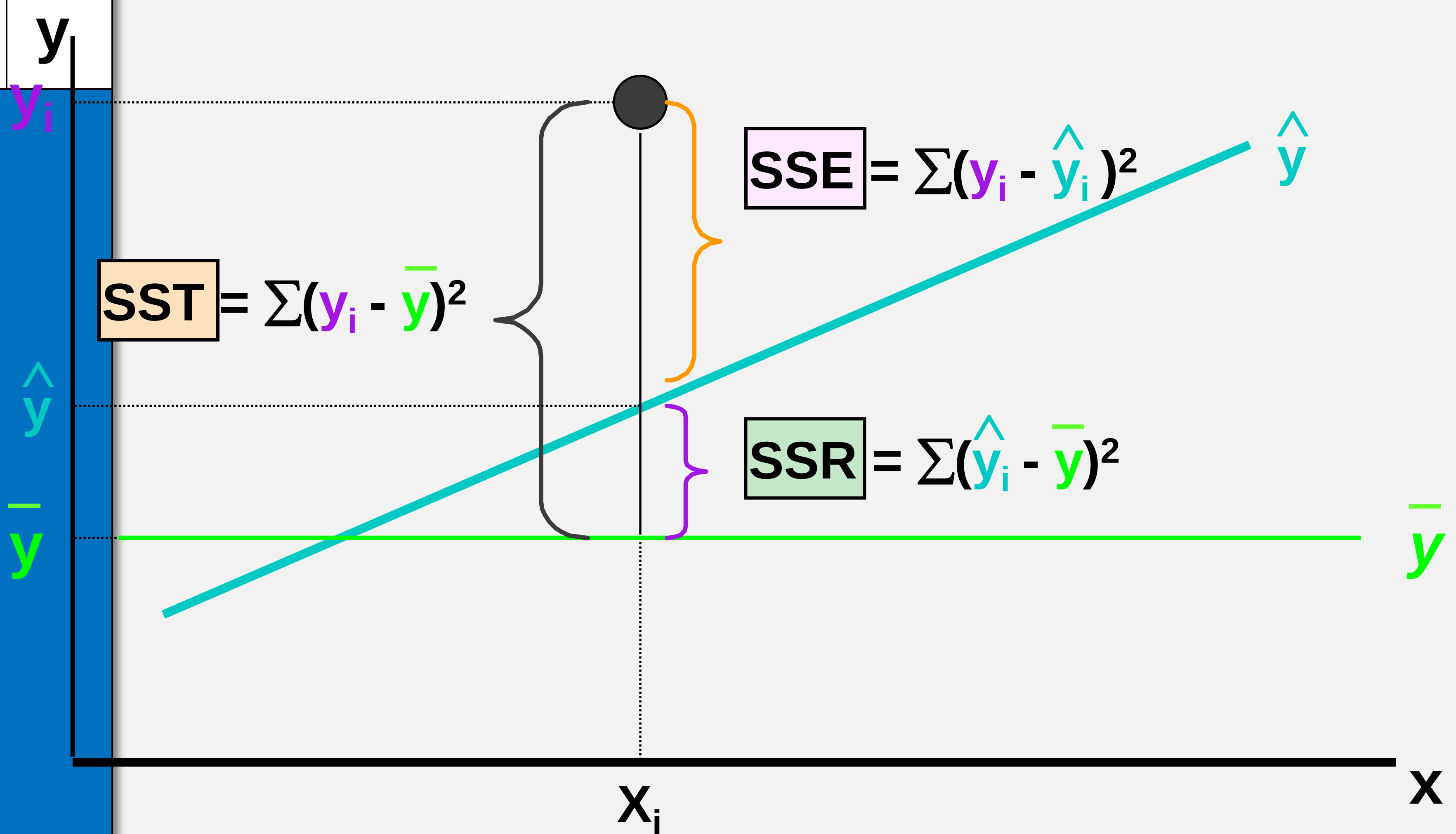
$\hat{y}$ = Estimated value of y for the given x value

(continued)

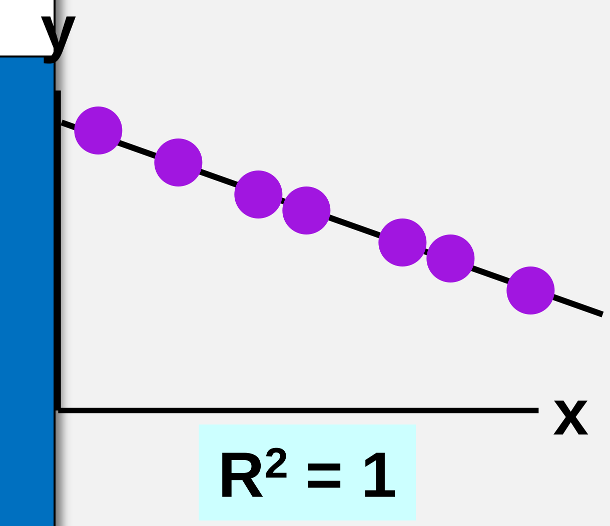
- SST = total sum of squares
  - Measures the variation of the y_i values around their mean y
- SSE = error sum of squares
  - Variation attributable to factors other than the relationship between x and y
- SSR = regression sum of squares
  - Explained variation attributable to the relationship between x and y

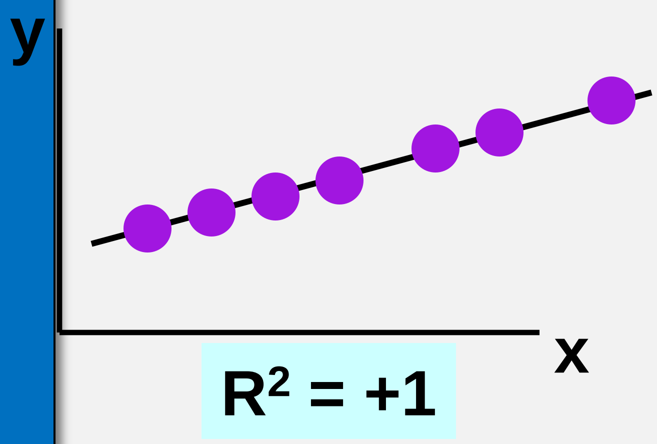
Explained and Unexplained Variation

(continued)



Examples of Approximate R^2 Values



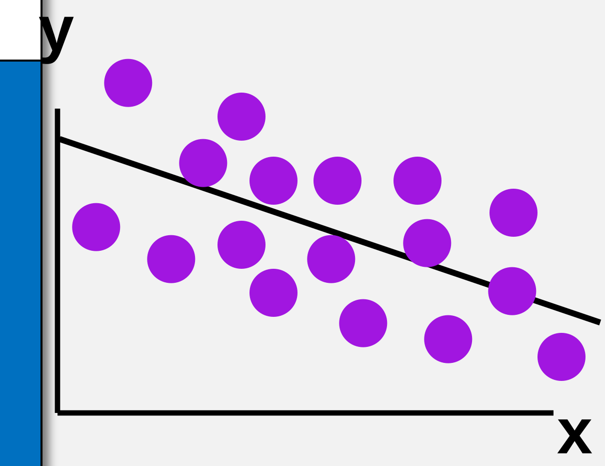


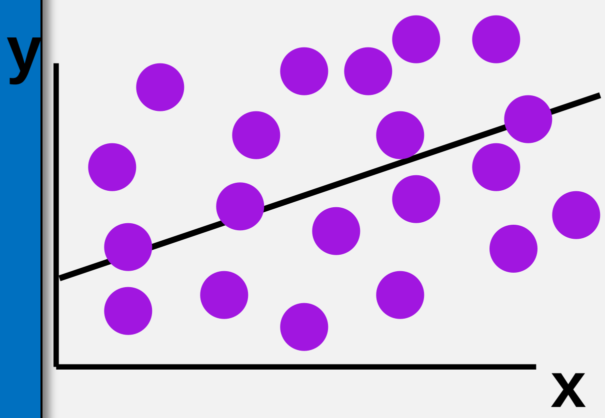
$R^2 = 1$

Perfect linear relationship between x and y:

100% of the variation in y is explained by variation in x

Examples of Approximate R^2 Values



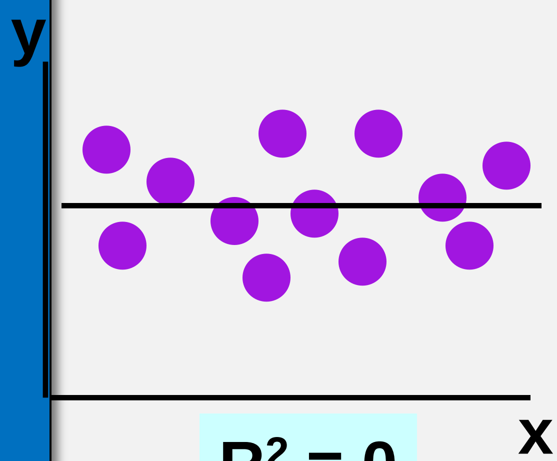


$0 < R^2 < 1$

Weaker linear relationship between x and y:

Some but not all of the variation in y is explained by variation in x

Examples of Approximate R^2 Values



$R^2 = 0$

$R^2 = 0$

No linear relationship between x and y:

The value of Y does not depend on x. (None of the variation in y is explained by variation in x)

Example

- What we will do with the data
  - Plot as a scatter plot
  - Assess mean for replicate data points

Reference Materials and Acknowledgements

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