Qualitative Analysis of Commom Cations in Water

Introduction to Analytical Chemistry

Chemistry is the study of matter, including its composition and structure, its physical properties, and its reactivity. There are many ways to study chemistry, but, we traditionally divide it into five fields: organic chemistry, inorganic chemistry, biochemistry, physical chemistry, and analytical chemistry. Although this division is historical and, perhaps, arbitrary—as witnessed by current interest in interdisciplinary areas such as bioanalytical chemistry and organometallic chemistry—these five fields remain the simplest division spanning the discipline of chemistry.

1. Chemical Principles

Background to chemical principles involved in the isolation and identification of cations from mixtures are described. Solubility of ionic compounds in water and the solubility variation by common ion effect, pH effect, coordination complex formation, and redox reaction are described in relation to the selective precipitation or dissolution of salts of the cations.

Solubility

Solution

The solution is a homogeneous mixture of two or more substances.

Solution related terminologies

- **Miscible** substances make a solution upon mixing with each other in any proportion. For example, ethanol and water are miscible to each other.
- **Immiscible** substances do not make solutions upon mixing in any proportion.
- **Partially miscible** substances can make a solution upon mixing up to a certain extent but not in all proportions.
- **A solvent** is a substance in a larger amount in the solution.
- **A solute** is a substance in a smaller amount in the solution.
- **An unsaturated solution** is a solution in which the solvent is holding solute less than the maximum limit, i.e., in which more solute can be dissolved.
- **A saturated solution** is a solution in which the solvent is holding the maximum amount of solute it can dissolve.

Water -a universal solvent

Water is one of the most important solvents because it is present all around us - it covers more than 70% of the earth and it is more than 60% of our body mass. Water is a polar molecule having a partial negative end on oxygen and a partially positive end on hydrogen atoms. that can dissolve most of the polar and ionic compounds. In ionic compounds, cations are held by anions through electrostatic interaction. When an ionic compound dissolves into water it dissociates into cations and anions, each surrounded by a layer of water molecules held by ion-dipole interactions. The water molecules around ions make ion-dipole interaction by orienting their partial negative end towards cations and their partial positive end towards anions. The energy needed to break ion-ion interaction in the ionic compounds is partially compensated by the energy released by establishing the ion-dipole interactions. The energy gained due to ion-dipole interactions and

nature's tendency to disperse is the driving forces responsible for the dissolution of ionic compounds.

Solubility

Solubility is the ability of a substance to form a solution with another substance.

The solubility of a solute in a specific solvent is quantitatively expressed as the concentration of the solute in the saturated solution. Usually, the solubility is tabulated in the units of grams of solute per 100 mL of solvent (g/100 mL). The solubility of ionic compounds in water varies over a wide range. All ionic compounds dissolve to some extent.

For practical purposes, a substance is considered **insoluble** when its solubility is less than 0.1 g per 100 mL of solvent.

For example, lead(II)iodide (PbI_2) and silver chloride ($AgCl$) are insoluble in water because the solubility of PbI_2 is 0.0016 mol/L of the solution and the solubility of $AgCl$ is about 1.3 x 10^{-5} mol/L of solution. Potassium iodide (KI) and $Pb(NO_3)_2$ are soluble in water. When aqueous solutions of KI and $Pb(NO_3)_2$ are mixed, the insoluble combination of ions, i.e., PbI_2 in this case, precipitates, as illustrated in Figure 1.1.1

Figure 1.1.1: Precipitation reaction: $Pb(NO_3)_2(aq)+2KI(aq)\longrightarrow PbI_2(s)\downarrow+2KNO_3(aq)$ source: PRHaney [CC BY-SA (https://creativecommons).

Solubility guidelines for dissolution of ionic compounds in water

There are no fail-proof guidelines for predicting the solubility of ionic compounds in water. However, the following guideline can predict the solubility of most ionic compounds.

Soluble ions

1. Salts of alkali metals (Li^+, Na^+, K^+, Rb^+, Cs^+) and ammonia (NH_4^+) are soluble. For example, NaClNaCl, and $(NH_4)_3PO_3$ are soluble.
2. Salts of nitrate (NO_3^-), acetate (CH_3COO^-), and perchlorate (ClO_4^-) are soluble. For example, $Pb(NO_3)_2$, and $Ca(CH_3COO)_2$ are soluble.
3. Salts of chloride (Cl^-), bromide (Br^-), and Iodide (I^-) are soluble, except when the cation is Lead (Pb^{2+}), Mercury (Hg_2^{2+}), or Silver (Ag^+). Remember the acronym "LMS" based on the first letter of the element name, or phrase 'Let Me See" to recall Lead, Mercury, and Silver.
4. Sulfates (SO_4^{2-}) are soluble except when the cation is, Pb^{2+}, Hg_2^{2+}, or Ag^+ (recall "Let Me See" for Lead, Mercury, and Silver) or a heavy alkaline earth metal ion: calcium (Ca^{2+}), barium (Ba^{2+}), or strontium (Sr^{2+}). (Remember the acronym "CBS" based on the first letter of the element name, or phrase "Come By Soon" to recall calcium, barium, and strontium.)

Insoluble ions

1. Hydroxide (OH^-) and sulfides (S^{2-}) are insoluble except when the cation is a heavy alkaline earth metal ion: Ca^{2+},Ba^{2+}, or Sr^{2+} (recall "Come By Soon" for calcium, barium, and strontium), alkali metals and ammonium. For example, $Mg(OH)_2$ and CuSCuS are insoluble.
2. Carbonates (CO_3^{2-}), phosphates (PO_4^{3-}), and oxide (O^{2-}) are insoluble except when the cation is an alkali metal ion or ammonium. For example, $CaCO_3$, and Fe_2O_3 are insoluble.
3. If there is a conflict between the two guidelines, then the guideline listed first has priority. For example, $CaCO_3$ is insoluble (rule#6), but Na_2CO_3 is soluble (rule#1 has priority over rule#6).

Precipitation reactions

Precipitation reactions are a class of chemical reactions in which two solutions are mixed and a solid product, called a precipitate, separates out. Precipitation reaction happening upon mixing solutions of ionic compounds in water can be predicted as illustrated in Figure 1.1.2 . The first step is to list the soluble ionic compounds and then cross-combine the cations of one with the anion of the other to make the potential products. If any of the potential products is an insoluble ionic compound, it will precipitate out. For example when NaOH solution is mixed with $MgCl_2$ solution, $Mg(OH)_2$ is a cross-combination that forms an insoluble compound, it will precipitate out.

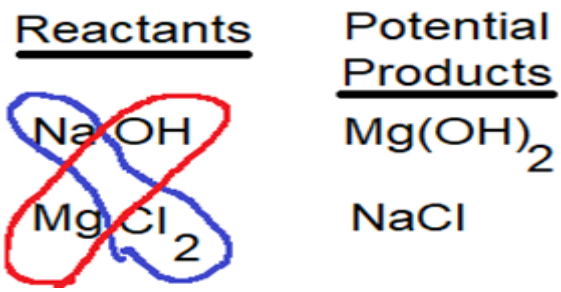


Figure 1.1.2 Cross-combine the cation-anion in the reactants. If any of the cross-combination is an insoluble salt, it will precipitate out, e.g: $NaOH(aq)+MgCl_2(aq)\longrightarrow Mg(OH)_2(s)\downarrow+NaCl(aq)$.

Figure 1.1.3 shows precipitates of some insoluble ionic compounds formed by mixing aqueous solutions of appropriate soluble ionic compounds.

Figure 1.1.3 The precipitates of some insoluble ionic compounds formed by mixing the aqueous solution of appropriate soluble ionic compounds. The precipitates are from left: white Calcium sulfate ($CaSO_4$), black Iron(II) hydroxide ($Fe(OH)_2$), brown Iron(III) hydroxide ($Fe(OH)_3$), and blue Copper(II) hydroxide ($Cu(OH)_2$). Note that the precipitate is not yet settled at the bottom of the solution, it is still in suspension form in these exemples

1.2: Solubility equilibria

Solubility product constant (K_{sp})

All ionic compounds dissolve in water to some extent. Ionic compounds are **strong electrolytes**, i.e., they dissociate completely into ions upon dissolution. When the amount of ionic compound added to the mixture is more than the solubility limit, the excess undissolved solute (solid) exists in equilibrium with its dissolved aqueous ions. For example, the following equation represents the equilibrium between solid $AgCl(s)$ and its dissolved $Ag^+(aq)$ and $Cl^+(aq)$ ions, where the subscript (s) means solid, i.e., the undissolved fraction of the compound, and (aq) means aqueous or dissolved in water.

$$\mathbf{AgCl(s) \rightleftharpoons Ag^+(aq) + Cl^-(aq)}$$

Like any other chemical equilibrium, this equilibrium has an equilibrium constant (K_{eq}) :

$$K_{eq} = [Ag^+][Cl^-]$$

Note that solid or pure liquid species do not appear in the equilibrium constant expression as the concentration in the solid or pure liquid remains constant. This equilibrium constant has a separate name **Solubility Product Constant** (K_{sp}) based on the fact that it is a product of the molar concentration of dissolved ions, raised to the power equal to their respective coefficients in the chemical equation, e.g.,

$$K_{sp} = [Ag^+][Cl^-] = 1.8 \times 10^{-10}$$

The solubility product constant (K_{sp}), is the equilibrium constant for an ionic compound dissolving in an aqueous solution.

Similarly, the dissolution equilibrium for $PbCl_2$ can be shown as:

$$PbCl_2(s) \rightleftharpoons Pb_2^+(aq) + 2\,Cl{-}(aq)$$

with

$$K_{sp} = [Pb^{2+}][Cl^-]^2 = 1.6 \times 10^{-5}$$

And the dissolution equilibrium for Hg_2Cl_2 is similar:

$$Hg_2Cl_2(s) \rightleftharpoons Hg_{22}^+(aq) + 2\,Cl{-}(aq)$$

with

$$K_{sp} = [Hg_2^{2+}][Cl^-]^2 = 1.3 \times 10^{-18}$$

Selective precipitation

Selective precipitation is a process involving adding a reagent that precipitates one of the dissolved cations or a particular group of dissolved cations but not the others.

According to solubility rule# 5, both Cu^{2+} and Ni^{2+} form insoluble salts with S^{2-}. However, the solubility of CuS and NiS differ enough that if an appropriate concentration of S^{2-} is maintained, CuS can be precipitated leaving Ni^{2+} dissolved. The following calculations based on the K_{sp} values prove it.

$$CuS(s) \rightleftharpoons Cu^{2+}(aq) + S^{2-}(aq), \quad K_{sp} = [Cu^{2+}][S^{2-}] = 8.7 \times 10^{-36}$$

$$NiS(s) \rightleftharpoons Ni^{2+}(aq) + S^{2-}(aq), \quad K_{sp} = [Ni^{2+}][S^{2-}] = 1.8 \times 10^{-21}$$

Molar concentration of sulfide ions $[S^{2-}]$, in moles/liter in a saturated solution of the ionic compound can be calculated by rearranging their respective K_{sp} expression, e.g. for CuS solution, $K_{sp}=[Cu^{2+}][S^{2-}]$ rearranges to:

$$[S^{2-}] = \frac{K_{sp}}{[Cu^{2+}]}$$

Assume Cu^{2+} is 0.1 M, plugging in the values in the above equation allow calculating the molar concentration of S^{2-} in the saturated solution of CuS:

$$[S^{2-}] = \frac{K_{sp}}{[Cu^{2+}]} = \frac{8.7 \times 10^{-36}}{0.1} = 8.7 \times 10^{-35}\text{-M}$$

Similar calculations show that the molar concentration of S^{2-} in the saturated solution of 0.1M NiS is 1.8×10^{-20} M. If S^{2-} concentration is kept more than 8.7×10^{-35} M but less than 1.8×10^{-20} M, CuS will selectively precipitate leaving Ni^{2+} dissolved in the solution.

Another example is the selective precipitation of Lead, silver, and mercury by adding HCl to the solution. According to rule# 3 of solubility of ionic compounds, chloride Cl^- forms soluble salt with the cations except with Lead (Pb^{2+}), Mercury (Hg_2^{2+}), or Silver (Ag^+). Adding HCl as a source of Cl^- in the solution will selectively precipitate lead (Pb^{2+}), mercury (Hg_2^{2+}), and silver (Ag^+), leaving other cations dissolved in the solution.

1.3: Varying solubility of ionic compounds

Le Chatelier's principle

Ionic compounds dissociate into ions when they dissolve in water. An equilibrium is established between ions in water and the undissolved compound. The solubility of the ionic compounds can be varied by stressing the equilibrium through changes in the concentration of the ions.

Le Chatelier's principle

Le Chatelier's principle can be stated as "when a system at equilibrium is subjected to a change in concentration, temperature, volume, or pressure, the system will change to a new equilibrium, such that the applied change is partially counteracted."

If the ions in the solubility equilibrium are increased or decreased by another reaction going on in parallel, the equilibrium will counteract by decreasing or increasing the solubility of the compound. This use of Le Chatelier's principle to vary the solubility of sparingly soluble ionic compounds is explained with examples in the following.

Common ion effect

Consider dissolution of a sparingly soluble ionic compound CaF_2 in water:

$$CaF_2(s) \rightleftharpoons Ca^{2+}(aq) + 2F^-(aq), \quad K_{sp} = [Ca^{2+}][F^-]^2 = 1.5 \times 10^{-10}$$

The solubility (S) can be expressed in the units of mol/L or molarity (M). Similarly, the concentration of any species in square brackets, as $[Ca^{2+}]$ in the above-mentioned K_{sp} expression, is also in the units of mol/L or M.

NaF is a water-soluble ionic compound that has F^- in common with the above equilibrium. The addition of NaF into the mixture will increase the concentration of F^- causing a decrease in the solubility of CaF_2 because the solubility equilibrium will move in the reverse direction to counteract the rise in the concentration of the common ion. This is called the **common ion effect**.

The common ion effect refers to the decrease in the solubility of a sparingly soluble ionic compound by adding a soluble ionic compound that has an ion in common with the sparingly soluble ionic compound.

A quantitative estimate of this common ion effect is given with the help of the following calculations. If solubility of CaF_2 in pure water is S mol/L, then [Ca^{2+}] = S, and [F^-] = 2S. Plugging in these values in the K_{sp} expression and rearranging shows that the solubility of CaF_2 in pure water is 3.3 x 10^{-4} M:

$$K_{sp} = [Ca^{2+}][F^-]^2$$

$$1.5 \times 10^{-10} = S(2S)^2$$

$$S = \sqrt[3]{1.5 \times 10^{-10}/4} = 3.310^{-4}\ \text{M}$$

If F^- concentration is raised to 0.1M by dissolving NaF in the solution, then the molar solubility of CaF_2 changes to a new value S_i , [Ca^{2+}] = S_i, and [F^-] = (0.1 + S_i) = 0.1 (S_i cancels because it is negligible compared to 0.1). Plugging in these values in the K_{sp} expression and rearranging shows that the new solubility (S_i) of CaF_2 is 1.5 x 10^{-5}M:

$$K_{sp} = [Ca^{2+}][F^-]^2$$

$$1.5 \times 10^{-10} = S_i(0.1)^2$$

$$S_i = \frac{1.5 \times 10^{-10}}{(0.1)^2} = 1.5 \times 10^{-8}\ \text{M}$$

It means the solubility of CaF_2 is decreased by more than twenty thousand times by the common ion effect described above.

Generally, the solubility of sparingly soluble ionic compounds decreases by adding a common ion to the equilibrium mixture.

An example of a common ion effect is in the separation of $PbCl_2$ from $AgCl$ and Hg_2Cl_2 precipitates. $PbCl$ is the most soluble in hot water among these three sparingly soluble compounds. So, $PbCl_2$ is selectively dissolved in hot water and separated. The solution is then cooled to room temperate and HCl is added to it as a source of common ion Cl^- to enforce re-precipitation of $PbCl_2$:

$$Pb^{2+}(aq) + 2\,Cl^-(aq) \rightleftharpoons PbCl_2(s)$$

Effect of pH

The pH is related to the concentration of H_3O^+ and OH^- in the solution. Increasing pH increases OH^- and decreases H_3O^+ concentration in the solution and decreasing pH has the opposite effect. If one of the ions in the solubility equilibrium of a sparingly soluble ionic compound is an acid or a base, its concentration will change with changes in the pH. It is because acids will neutralize with OH^- at high pH and bases will neutralize with H_3O^+ at low pH. For example, consider the dissolution of $Mg(OH)_2$ in pure water.

$$Mg(OH)_2(s) \rightleftharpoons Mg^{2+}(aq) + 2\,OH^-(aq), \quad K_{sp} = [Mg^{2+}][OH^-]^2 = 2.1 \times 10^{-13}$$

Making the solution acidic, i.e., a decrease in pH adds more H_3O^+ ion that removes OH^- by the following neutralization reaction.

$$H_3O^+(aq) + OH^-(aq) \rightleftharpoons 2\,H_2O(l)$$

According to Le Chatelier's principle, the system moves in the forward direction to make up for the loss of OH^-. In other words, $Mg(OH)_2$ is insoluble in neutral or alkaline water and becomes soluble in acidic water.

Generally, the solubility of an ionic compound containing basic anion increases by decreasing pH, i.e., in an acidic medium.

Generally, the solubility of an ionic compound containing basic anion increases by decreasing pH, i.e., in an acidic medium.

In a qualitative analysis of cations, dissociation of H_2S is used as a source of S^{2-} ions:

$$H_2S(g) + 2\,H_2O(l) \rightleftharpoons 2\,H_3O^+(aq) + S^{2-}(aq)$$

The reaction is pH-dependent, i.e., the extent of dissociation of H_2S can be decreased by adding HCl as a source of common ion H_3O^+ or increased by adding a base as a source of OH^- that removes H_3O^+ from the products:

$$OH^-(aq) + H_3O^+(aq) \rightleftharpoons 2\,H_2O(l)$$

Generally, the solubility of weak acids can be increased by increasing the pH and decreased by decreasing the pH. The opposite is true for the weak bases.

Complex ion equilibria

Transition metal ions, like Ag^+, Cu^{2+}, Ni^{2+}, etc. tend to be strong Lewis acids, i.e., they can accept a lone pair of electrons from Lewis bases. Neutral or anionic species with a lone pair of electrons, like $H_2\ddot{O}:$, $:NH_3$, $:\bar{C}N:$, $:\ddot{\underset{\cdot\cdot}{Cl}}:^-$, etc. can act as Lewis bases in these reactions. The bond formed by the donation of a lone pair of electrons of a Lewis base to a Lewis acid is called a **coordinate covalent bond**. The neutral compound or ion that results from the Lewis acid-base reaction is called a **coordination complex** or a **complex ion**. For example, silver ion dissolved in water is often written as $Ag^+_{(aq)}$, but, in reality, it exists as complex ion $Ag(H_2O)_2^+$ in which Ag^+ accepts lone pair of electrons from oxygen atoms in water molecules. Transition metal ion in a coordination complex or complex ion, e.g., Ag^+ in $Ag(H_2O)_2^+$ is called **central metal ion** and the Lewis base like $H_2\ddot{O}:$, in $Ag(H_2O)_2^+$, is called a **ligand**. The strength of a ligand is the ability of a ligand to donate its lone pair of electrons to a central metal ion. If a stronger ligand is added to the solution, it displaces a weaker ligand. For example, if $:NH_3$ is dissolved in the solution containing $Ag(H_2O)_2^+$, the $:NH_3$ displaces $H_2\ddot{O}:$ from the complex ion:

$$Ag(H_2O)_2^+(aq) + 2\,NH_3(aq) \rightleftharpoons Ag(NH_3)_2^+(aq) + 2\,H_2O(aq)$$

Equilibrium constant for the formation of complex ion is called **formation constant** (K_f), e.g, in the case of above reaction:

$$K_f = \frac{[Ag(NH_3)_2^+]}{[Ag^+] \times [NH_3]^2} = 1.7 \times 10^7$$

Large value of K_f in the above reaction shows that the reaction is highly favored in the forward direction. If ammonia is present in water, it increases the solubility of $AgCl$ by removing the Ag^+ ion from the products, just like acid (H_3O^+) increases the solubility of $Mg(OH)_2$ by removing OH^- from the products:

$$AgCl(s) \rightleftharpoons Ag^+(aq) + Cl^-(aq) \quad K_f = 1.8 \times 10^{-10}$$

$$Ag^+(aq) + 2\,NH_3(aq) \rightleftharpoons Ag(NH_3)_2^+(aq) \quad K_f = 1.7 \times 10^7$$

Adding above reactions: $AgCl(aq) + 2\,NH_3(aq) \rightleftharpoons Ag(NH_3)_2^+(aq) + Cl^-(aq) \quad K = 3.0 \times 10^{-3}$

The equilibrium constant for the dissolution of $AgCl(s)$ changes from 1.8 x 10^{-10} in pure water to 3.0 x 10^{-3} in the water containing dissolved ammonia, i.e., a 17 million times increase. It makes insoluble $AgCl(s)$ quite soluble. This reaction is used to separate silver ions from mercury ions in a mixture of $AgCl$ and Hg_2Cl_2 mixture precipitates.

Generally, the solubility of metal compounds containing metals capable of coordinate complex formation increases by adding a strong ligand to the solution.

Manipulating chemical equations

The chemical equations can be manipulated like algebraic equations, i.e., they can be multiplied or divided by a constant, added, and subtracted, as demonstrated in the example of the silver ammonia complex formation reactions shown above. Note that the species on the right side of the equation cancel the same species on the left side of any other equation like algebraic equations, e.g., Ag^+ is canceled the final equation.

When two equilibrium reactions are added, their equilibrium constants are multiplied to get the equilibrium constant of the overall reaction, i.e, $K = K_{sp} \times K_f$ in the above reactions.

Redox reactions

There are three major types of chemical reactions, precipitation reactions, acid-base reactions, and redox reactions.

Precipitation reactions

Precipitation reactions of ionic compounds are double replacement reactions where the cation of one compound combines with the anion of another and vice versa, such that one of the new combinations is an insoluble salt.

For example, when silver nitrate ($AgNO_3$) solution is mixed with sodium chloride ($NaCl$) solution, an insoluble compound silver chloride ($AgCl$) precipitates out of the solution:

$$AgNO_3(aq) + HCl(aq) \longrightarrow AgCl(s)\downarrow + NaNO_3(aq)$$

Acid-base reactions

Acid-base reactions are the reactions involving the transfer of a proton.

For example, H_2S dissociates in water by donating its proton to water molecules:

$$H_2S(g) + 2\,H_2O(l) \rightleftharpoons 2\,H_3O^+(aq) + S^{2-}(aq)$$

Redox reactions

Redox reactions are the reactions involving the transfer of electrons.

For example, when sodium metal (Na) reacts with chlorine gas (Cl_2), sodium loses electrons and becomes Na^+ cation and chlorine gains electrons and becomes Cl^- anion that combine to form NaCl salt:

$$2\,Na(s) + 2\,Cl_2(g) \longrightarrow NaCl(s)$$

An example of a redox reaction in qualitative analysis of cations is the dissolution of NiS precipitate by adding an oxidizing acid HNO_3. The S^{2-} is a weak base that can be removed from the product by adding a strong acid like HCl:

$$S^{2-}(aq) + 2\,H_3O^+(aq) \rightleftharpoons H_2S(aq) + 2\,H_2O(l)$$

Therefore, addition of HCl is sufficient to dissolve FS precipitate by removal of S^{2-} from the products:

$$FeS(s) + 2\,H_3O^+(aq) \rightleftharpoons Fe^{2+}(aq) + H_2S(aq) + 2\,H_2O(l)$$

However, the addition of HCl does not remove S^{2-} sufficient enough to dissolve a relatively less soluble NiS precipitate. Nitric acid (HNO_3) that is a source of an oxidizing agent NO_3^- is needed to remove S^{2-} to a higher extent for dissolving NiS:

$$3\,S^{2-}(aq) + 2\,NO_3^-(aq) + 8\,H_3O^+(aq) \longrightarrow 3\,S(s, yellow)\downarrow + 2\,NO(g)\uparrow + 12\,H_2O(l)$$

In this reaction, sulfur is oxidized from an oxidation state of -2 in S^{2-} to an oxidation state of zero in S, and nitrogen is reduced from an oxidation state of +5 in NO_3^- to an oxidation state of +2 in NO.

Analytical Chemistry 2.1 (Harvey)

Basic Tools of Analytical Chemistry

2.1: Measurements in Analytical Chemistry

Units of Measurement

A measurement usually consists of a unit and a number that expresses the quantity of that unit. We can express the same physical measurement with different units, which creates confusion if we are not careful to specify the unit. For example, the mass of a sample that weighs 1.5 g is equivalent to 0.0033 lb or to 0.053 oz. To ensure consistency, and to avoid problems, scientists use the common set of fundamental base units listed in Table 2.1.1 . These units are called SI units after the Système International d'Unités.

It is important for scientists to agree upon a common set of units. In 1999, for example, NASA lost a Mar's Orbiter spacecraft because one engineering team used English units in their calculations and another engineering team used metric units. As a result, the spacecraft came too close to the planet's surface, causing its propulsion system to overheat and fail.

Some measurements, such as absorbance, do not have units. Because the meaning of a unitless number often is unclear, some authors include an artificial unit. It is not unusual

to see the abbreviation AU—short for absorbance unit—following an absorbance value, which helps clarify that the measurement is an absorbance value.

Table 2.1.1 : Fundamental Base SI Units

Measurement	Unit	Symbol	Definition (1 unit is...)
mass	kilogram	kg	...the mass of the international prototype, a Pt-Ir object housed at the Bureau International de Poids and Measures at Sèvres, France. (Note: The mass of the international prototype changes at a rate of approximately 1 µg per year due to reversible surface contamination. The reference mass, therefore, is determined immediately after its cleaning using a specified procedure. Current plans call for retiring the international prototype and defining the kilogram in terms of Planck's constant; see this link for more details.)
distance	meter	m	...the distance light travels in $(299\ 792\ 458)^{-1}$ seconds.
temperature	Kelvin	K	...equal to $(273.16)^{-1}$, where 273.16 K is the triple point of water (where its solid, liquid, and gaseous forms are in equilibrium).
time	second	s	...the time it takes for 9 192 631 770 periods of radiation corresponding to a specific transition of the ^{133}Cs atom.
current	ampere	A	...the current producing a force of 2×10^{-7} N/m between two straight parallel conductors of infinite length separated by one meter (in a vacuum).
amount of substance	mole	mol	...the amount of a substance containing as many particles as there are atoms in exactly 0.012 kilogram of ^{12}C.
light	candela	cd	...the luminous intensity of a source with a monochromatic frequency of 540×10^{12} hertz and a radiant power of $(683)^{-1}$ watts per steradian.

There is some disagreement on the use of "amount of substance" to describe the measurement for which the mole is the base SI unit; see "What's in a Name? Amount of Substance, Chemical Amount, and Stoichiometric Amount," the full reference for which is Giunta, C. J. *J. Chem. Educ.* **2016**, *93*, 583–586.

We define other measurements using these fundamental SI units. For example, we measure the quantity of heat produced during a chemical reaction in joules, (J), where 1 J is equivalent to 1 m kg/s . Table 2.1.2 provides a list of some important derived SI units, as well as a few common non-SI units.

Table 2.1.2 : Derived SI Units and Non-SI Units of Importance to Analytical Chemistry

Measurement	Unit	Symbol	Equivalent SI Units
length	angstrom (non-SI)	Å	$1\ Å = 1 \times 10^{-10}$ m
volume	liter (non-SI)	L	$1\ L = 10^{-3}\ m^3$
force	newton (SI)	N	$1\ N = 1\ m{\cdot}kg/s^2$
pressure	pascal (SI)	Pa	$1\ Pa = 1\ N/m^3 = 1\ kg/(m{\cdot}s^2)$
	atmosphere (non-SI)	atm	1 atm = 101 325 Pa
energy, work, heat	joule (SI)	J	$1\ J = 1\ N{\cdot}m = 1\ m^2{\cdot}kg/s^2$
	calorie (non-SI)	cal	1 cal = 4.184 J
	electron volt (non-SI)	eV	$1\ eV = 1.602\ 177\ 33 \times 10^{-19}$ J
power	watt (SI)	W	$1\ W = 1\ J/s = 1\ m^2{\cdot}kg/s^3$
charge	coulomb (SI)	C	$1\ C = 1\ A{\cdot}s$
potential	volt (SI)	V	$1\ V = 1\ W/A = 1\ m^2{\cdot}kg/(s^3{\cdot}A)$
frequency	hertz (SI)	Hz	$1\ Hz = s^{-1}$
temperature	Celcius (non-SI)	°C	°C = K – 273.15

Chemists frequently work with measurements that are very large or very small. A mole contains 602 213 670 000 000 000 000 000 particles and some analytical techniques can detect as little as 0.000 000 000 000 001 g of a compound. For simplicity, we express these measurements using scientific notation; thus, a mole contains $6.022\ 136\ 7 \times 10^{23}$ particles, and the detected mass is 1×10^{-15} g. Sometimes we wish to express a measurement without the exponential term, replacing it with a prefix (Table 2.1.3). A mass of 1×10^{-15} g, for example, is the same as 1 fg, or femtogram.

> Writing a lengthy number with spaces instead of commas may strike you as unusual. For a number with more than four digits on either side of the decimal point, however, the recommendation from the International Union of Pure and Applied Chemistry is to use a thin space instead of a comma.

Table 2.1.3 : Common Prefixes for Exponential Notation

Prefix	Symbol	Factor	Prefix	Symbol	Factor	Prefix	Symbol	Factor
yotta	Y	10^{24}	kilo	k	10^{3}	micro	μ	10^{-6}
zetta	Z	10^{21}	hecto	h	10^{2}	nano	n	10^{-9}
eta	E	10^{18}	deka	da	10^{1}	pico	p	10^{-12}
peta	P	10^{15}	—	—	10^{0}	femto	f	10^{-15}
tera	T	10^{12}	deci	d	10^{-1}	atto	a	10^{-18}
giga	G	10^{9}	centi	c	10^{-2}	zepto	z	10^{-21}
mega	M	10^{6}	milli	m	10^{-3}	yocto	y	10^{-24}

Uncertainty in Measurements

A measurement provides information about both its magnitude and its uncertainty. Consider, for example, the three photos in Figure 2.1.1 , taken at intervals of approximately 1 sec after placing a sample on the balance. Assuming the balance is properly calibrated, we are certain that the sample's mass is more than 0.5729 g and less than 0.5731 g. We are uncertain, however, about the sample's mass in the last decimal place since the final two decimal places fluctuate between 29, 30, and 31. The best we can do is to report the sample's mass as 0.5730 g ± 0.0001 g, indicating both its magnitude and its absolute uncertainty.

Figure 2.1.1 : When weighing an sample on a balance, the measurement fluctuates in the final decimal place. We record this sample's mass as 0.5730 g ± 0.0001

Significant Figures

A measurement's significant figures convey information about a measurement's magnitude and uncertainty. The number of significant figures in a measurement is the number of digits known exactly plus one digit whose value is uncertain. The mass shown in Figure 2.1.1 , for example, has four significant figures, three which we know exactly and one, the last, which is uncertain.

Suppose we weigh a second sample, using the same balance, and obtain a mass of 0.0990 g. Does this measurement have 3, 4, or 5 significant figures? The zero in the last decimal place is the one uncertain digit and is significant. The other two zeros, however, simply indicates the decimal point's location. Writing the measurement in scientific notation, 9.90×10^{-2}, clarifies that there are three significant figures in 0.0990.

> In the measurement 0.0990 g, the zero in green is a significant digit and the zeros in red are not significant digits.

✔ Example 2.1.1

How many significant figures are in each of the following measurements? Convert each measurement to its equivalent scientific notation or decimal form.

a. 0.0120 mol HCl
b. 605.3 mg $CaCO_3$
c. 1.043×10^{-4} mol Ag^+
d. 9.3×10^{4} mg NaOH

Solution

(a) Three significant figures; 1.20×10^{-2} mol HCl.

(b) Four significant figures; 6.053×10^{2} mg $CaCO_3$.

(c) Four significant figures; 0.000 104 3 mol Ag^+.

(d) Two significant figures; 93 000 mg NaOH.

There are two special cases when determining the number of significant figures in a measurement. For a measurement given as a logarithm, such as pH, the number of significant figures is equal to the number of digits to the right of the decimal point. Digits to the left of the decimal point are not significant figures since they indicate only the power of 10. A pH of 2.45, therefore, contains two significant figures.

> The log of 2.8×10^2 is 2.45. The log of 2.8 is 0.45 and the log of 10^2 is 2. The 2 in 2.45, therefore, only indicates the power of 10 and is not a significant digit.

An exact number, such as a stoichiometric coefficient, has an infinite number of significant figures. A mole of $CaCl_2$, for example, contains exactly two moles of chloride ions and one mole of calcium ions. Another example of an exact number is the relationship between some units. There are, for example, exactly 1000 mL in 1 L. Both the 1 and the 1000 have an infinite number of significant figures.

Using the correct number of significant figures is important because it tells other scientists about the uncertainty of your measurements. Suppose you weigh a sample on a balance that measures mass to the nearest ±0.1 mg. Reporting the sample's mass as 1.762 g instead of 1.7623 g is incorrect because it does not convey properly the measurement's uncertainty. Reporting the sample's mass as 1.76231 g also is incorrect because it falsely suggests an uncertainty of ±0.01 mg.

Significant Figures in Calculations

Significant figures are also important because they guide us when reporting the result of an analysis. When we calculate a result, the answer cannot be more certain than the least certain measurement in the analysis. Rounding an answer to the correct number of significant figures is important.

For addition and subtraction, we round the answer to the last decimal place in common for each measurement in the calculation. The exact sum of 135.621, 97.33, and 21.2163 is 254.1673. Since the last decimal place common to all three numbers is the hundredth's place

$$\begin{array}{r} 135.621 \\ 97.33 \\ 21.2163 \\ \hline 254.1673 \end{array}$$

we round the result to 254.17.

> The last common decimal place shared by 135.621, 97.33, and 21.2163 is shown in red.

For multiplication and division, we round the answer to the same number of significant figures as the measurement with the fewest number of significant figures. For example, when we divide the product of 22.91 and 0.152 by 16.302, we report the answer as 0.214 (three significant figures) because 0.152 has the fewest number of significant figures.

$$\frac{22.91 \times 0.152}{16.302} = 0.2136 = 0.214$$

There is no need to convert measurements in scientific notation to a common exponent when multiplying or dividing.

> It is important to recognize that the rules presented here for working with significant figures are generalizations. What actually is conserved is uncertainty, not the number of significant figures. For example, the following calculation
>
> $$101/99 = 1.02$$
>
> is correct even though it violates the general rules outlined earlier. Since the relative uncertainty in each measurement is approximately 1% (101 ± 1 and 99 ± 1), the relative uncertainty in the final answer also is approximately 1%. Reporting the answer as 1.0 (two significant figures), as required by the general rules, implies a relative uncertainty of 10%, which is too large. The correct answer, with three significant figures, yields the expected relative uncertainty. Chapter 4 presents a more thorough treatment of uncertainty and its importance in reporting the result of an analysis.

Finally, to avoid "round-off" errors, it is a good idea to retain at least one extra significant figure throughout any calculation. Better yet, invest in a good scientific calculator that allows you to perform lengthy calculations without the need to record intermediate values. When your calculation is complete, round the answer to the correct number of significant figures using the following simple rules.

1. Retain the least significant figure if it and the digits that follow are less than halfway to the next higher digit. For example, rounding 12.442 to the nearest tenth gives 12.4 since 0.442 is less than half way between 0.400 and 0.500.
2. Increase the least significant figure by 1 if it and the digits that follow are more than halfway to the next higher digit. For example, rounding 12.476 to the nearest tenth gives 12.5 since 0.476 is more than halfway between 0.400 and 0.500.
3. If the least significant figure and the digits that follow are exactly halfway to the next higher digit, then round the least significant figure to the nearest even number. For example, rounding 12.450 to the nearest tenth gives 12.4, while rounding 12.550 to the nearest tenth gives 12.6. Rounding in this manner ensures that we round up as often as we round down.

? Exercise 2.1.1

For a problem that involves both addition and/or subtraction, and multiplication and/or division, be sure to account for significant figures at each step of the calculation. With this in mind, report the result of this calculation to the correct number of significant figures.

$$\frac{0.250 \times (9.93 \times 10^{-3}) - 0.100 \times (1.927 \times 10^{-2})}{9.93 \times 10^{-3} + 1.927 \times 10^{-2}} =$$

Answer

The correct answer to this exercise is 1.9×10^{-2}. To see why this is correct, let's work through the problem in a series of steps. Here is the original problem

$$\frac{0.250 \times (9.93 \times 10^{-3}) - 0.100 \times (1.927 \times 10^{-2})}{9.93 \times 10^{-3} + 1.927 \times 10^{-2}} =$$

Following the correct order of operations we first complete the two multiplications in the numerator. In each case the answer has three significant figures, although we retain an extra digit, highlight in red, to avoid round-off errors.

$$\frac{2.482 \times 10^{-3} - 1.927 \times 10^{-3}}{9.93 \times 10^{-3} + 1.927 \times 10^{-2}} =$$

Completing the subtraction in the numerator leaves us with two significant figures since the last significant digit for each value is in the hundredths place.

$$\frac{0.555 \times 10^{-3}}{9.93 \times 10^{-3} + 1.927 \times 10^{-2}} =$$

The two values in the denominator have different exponents. Because we are adding together these values, we first rewrite them using a common exponent.

$$\frac{0.555 \times 10^{-3}}{0.993 \times 10^{-2} + 1.927 \times 10^{-2}} =$$

The sum in the denominator has four significant figures since each of the addends has three decimal places.

$$\frac{0.555 \times 10^{-3}}{2.920 \times 10^{-2}} =$$

Finally, we complete the division, which leaves us with a result having two significant figures.

$$\frac{0.555 \times 10^{-3}}{2.920 \times 10^{-2}} = 1.9 \times 10^{-2}$$

2.2: Concentration

Concentration is a general measurement unit that reports the amount of solute present in a known amount of solution

$$\text{concentration} = \frac{\text{amount of solute}}{\text{amount of solution}} \tag{2.2.1}$$

Although we associate the terms "solute" and "solution" with liquid samples, we can extend their use to gas-phase and solid-phase samples as well. Table 2.2.1 lists the most common units of concentration.

Table 2.2.1 : Common Units for Reporting Concentration

Name	Units	Symbol
molarity	$\frac{\text{moles solute}}{\text{liters solution}}$	M
formality	$\frac{\text{moles solute}}{\text{liters solution}}$	F
normality	$\frac{\text{equivalents solute}}{\text{liters solution}}$	N
molality	$\frac{\text{moles solute}}{\text{kilograms solvent}}$	m
weight percent	$\frac{\text{grams solute}}{\text{100 grams solution}}$	% w/w
volume percent	$\frac{\text{mL solute}}{\text{100 mL solution}}$	% v/v
weight-to-volume percent	$\frac{\text{grams solute}}{\text{100 mL solution}}$	% w/v
parts per million	$\frac{\text{grams solute}}{10^6 \text{ grams solution}}$	ppm
parts per billion	$\frac{\text{grams solute}}{10^9 \text{ grams solution}}$	ppb

> An alternative expression for weight percent is
>
> $$\frac{\text{grams solute}}{\text{grams solution}} \times 100$$
>
> You can use similar alternative expressions for volume percent and for weight-to-volume percent.

Molarity and Formality

Both molarity and formality express concentration as moles of solute per liter of solution; however, there is a subtle difference between them. ***Molarity*** is the concentration of a particular chemical species. ***Formality***, on the other hand, is a substance's total concentration without regard to its specific chemical form. There is no difference between a compound's molarity and formality if it dissolves without dissociating into ions. The formal concentration of a solution of glucose, for example, is the same as its molarity.

For a compound that ionizes in solution, such as $CaCl_2$, molarity and formality are different. When we dissolve 0.1 moles of $CaCl_2$ in 1 L of water, the solution contains 0.1 moles of Ca^{2+} and 0.2 moles of Cl^-. The molarity of $CaCl_2$, therefore, is zero since there is no undissociated $CaCl_2$ in solution; instead, the solution is 0.1 M in Ca^{2+} and 0.2 M in Cl^-. The formality of $CaCl_2$, however, is 0.1 F since it represents the total amount of $CaCl_2$ in solution. This more rigorous definition of molarity, for better or worse, largely is ignored in the current literature, as it is in this textbook. When we state that a solution is 0.1 M $CaCl_2$ we understand it to consist of Ca^{2+} and Cl^- ions. We will reserve the unit of formality to situations where it provides a clearer description of solution chemistry.

Molarity is used so frequently that we use a symbolic notation to simplify its expression in equations and in writing. Square brackets around a species indicate that we are referring to that species' molarity. Thus, $[Ca^{2+}]$ is read as "the molarity of calcium ions."

> For a solute that dissolves without undergoing ionization, molarity and formality have the same value. A solution that is 0.0259 M in glucose, for example, is 0.0259 F in glucose as well.

Normality

Normality is a concentration unit that no longer is in common use; however, because you may encounter normality in older handbooks of analytical methods, it is helpful to understand its meaning. ***Normality*** defines concentration in terms of an equivalent, which is the amount of one chemical species that reacts stoichiometrically with another chemical species. Note that this definition makes an equivalent, and thus normality, a function of the chemical reaction in which the species participates. Although a solution of H_2SO_4 has a fixed molarity, its normality depends on how it reacts. You will find a more detailed treatment of normality in Appendix 1.

> One handbook that still uses normality is *Standard Methods for the Examination of Water and Wastewater*, a joint publication of the American Public Health Association, the American Water Works Association, and the Water Environment Federation. This handbook is one of the primary resources for the environmental analysis of water and wastewater.

Molality

Molality is used in thermodynamic calculations where a temperature independent unit of concentration is needed. Molarity is based on the volume of solution that contains the solute. Since density is a temperature dependent property, a solution's volume, and thus its molar concentration, changes with temperature. By using the solvent's mass in place of the solution's volume, the resulting concentration becomes independent of temperature.

Weight, Volume, and Weight-to-Volume Percent

Weight percent (% w/w), ***volume percent*** (% v/v) and ***weight-to-volume percent*** (% w/v) express concentration as the units of solute present in 100 units of solution. A solution that is 1.5% w/v NH_4NO_3, for example, contains 1.5 gram of NH_4NO_3 in 100 mL of solution.

Parts Per Million and Parts Per Billion

Parts per million (ppm) and ***parts per billion*** (ppb) are ratios that give the grams of solute in, respectively, one million or one billion grams of sample. For example, a sample of steel that is 450 ppm in Mn contains 450 µg of Mn for every gram of steel. If we approximate the density of an aqueous solution as 1.00 g/mL, then we can express solution concentrations in ppm or ppb using the following relationships.

$$\text{ppm} = \frac{\mu\text{g}}{\text{g}} = \frac{\text{mg}}{\text{L}} = \frac{\mu\text{g}}{\text{mL}} \quad \text{ppb} = \frac{\text{ng}}{\text{g}} = \frac{\mu\text{g}}{\text{L}} = \frac{\text{ng}}{\text{mL}}$$

For gases a part per million usually is expressed as a volume ratio; for example, a helium concentration of 6.3 ppm means that one liter of air contains 6.3 µL of He.

> You should be careful when using parts per million and parts per billion to express the concentration of an aqueous solute. The difference between a solute's concentration in mg/L and ng/g, for example, is significant if the solution's density is not 1.00 g/mL. For this reason many organizations advise against using the abbreviation ppm and ppb (see section 7.10.3 at www.nist.gov). If in doubt, include the exact units, such as 0.53 µg Pb^{2+}/L for the concentration of lead in a sample of seawater.

Converting Between Concentration Units

The most common ways to express concentration in analytical chemistry are molarity, weight percent, volume percent, weight-to-volume percent, parts per million and parts per billion. The general definition of concentration in Equation 2.2.1 makes it is easy to convert between concentration units.

✔ Example 2.2.1

A concentrated solution of ammonia is 28.0% w/w NH_3 and has a density of 0.899 g/mL. What is the molar concentration of NH_3 in this solution?

Solution

$$\frac{28.0\text{ g NH}_3}{100\text{ g soln}} \times \frac{0.899\text{ g soln}}{\text{ml soln}} \times \frac{1\text{ mol NH}_3}{17.03\text{ g NH}_3} \times \frac{1000\text{mL}}{\text{L}} = 14.8\text{ M}$$

✔ Example 2.2.2

The maximum permissible concentration of chloride ion in a municipal drinking water supply is 2.50×10^2 ppm Cl^-. When the supply of water exceeds this limit it often has a distinctive salty taste. What is the equivalent molar concentration of Cl^-?

Solution

$$\frac{2.50 \times 10^2\text{ mg Cl}^-}{\text{L}} \times \frac{1\text{ g}}{1000\text{ mg}} \times \frac{1\text{ mol Cl}^-}{35.453\text{ gCl}^-} = 7.05 \times 10^{-3}\text{ M}$$

? Exercise 2.2.1

Which solution—0.50 M NaCl or 0.25 M $SrCl_2$—has the larger concentration when expressed in mg/mL?

Answer

The concentrations of the two solutions are

$$\frac{0.50\text{ mol NaCl}}{\text{L}} \times \frac{58.44\text{ g NaCl}}{\text{mol NaCl}} \times \frac{10^6\ \mu\text{g}}{\text{g}} \times \frac{1\text{L}}{1000\text{ mL}} = 2.9 \times 10^4\ \mu\text{g/mL NaCl}$$

$$\frac{0.25\text{ mol SrCl}_2}{\text{L}} \times \frac{158.5\text{ g SrCl}_2}{\text{mol SrCl}_2} \times \frac{10^6\ \mu\text{g}}{\text{g}} \times \frac{1\text{L}}{1000\text{ mL}} = 4.0 \times 10^4\ \mu\text{g/ml SrCl}_2$$

The solution of $SrCl_2$ has the larger concentration when it is expressed in µg/mL instead of in mol/L.

p-Functions

Sometimes it is inconvenient to use the concentration units in Table 2.2.1 . For example, during a chemical reaction a species' concentration may change by many orders of magnitude. If we want to display the reaction's progress graphically we might wish to plot the reactant's concentration as a function of the volume of a reagent added to the reaction. Such is the case in Figure 2.2.1 for the titration of HCl with NaOH. The *y*-axis on the left-side of the figure displays the $[H^+]$ as a function of the volume of NaOH. The initial $[H^+]$ is 0.10 M and its concentration after adding 80 mL of NaOH is 4.3×10^{-13}M. We easily can follow the change in $[H^+]$ for the addition of the first 50 mL of NaOH; however, for the remaining volumes of NaOH the change in $[H^+]$ is too small to see.

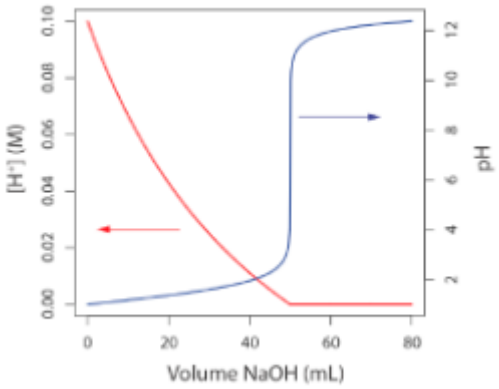


Figure 2.2.1 : Two curves showing the progress of a titration of 50.0 mL of 0.10 M HCl with 0.10 M NaOH. The $[H^+]$ is shown on the left *y*-axis and the pH on the right *y*-axis.

When working with concentrations that span many orders of magnitude, it often is more convenient to express concentration using a p-function. The p-function of X is written as pX and is defined as

$$pX = -\log(X)$$

The pH of a solution that is 0.10 M H^+ for example, is

$$pH = -\log[H^+] = -\log(0.10) = 1.00$$

and the pH of 4.3×10^{-13} M H^+ is

$$pH = -\log[H^+] = -\log(4.3 \times 10^{-13}) = 12.37$$

Figure 2.2.1 shows that plotting pH as a function of the volume of NaOH provides more useful information about how the concentration of H^+ changes during the titration.

> A more appropriate equation for pH is $pH = -\log(a_{H^+})$ where a_{H^+} is the activity of the hydrogen ion. See Chapter 6.9 for more details. For now the approximate equation $pH = -\log[H^+]$ is sufficient.

✔ Example 2.2.3

What is pNa for a solution of 1.76×10^{-3} M Na_3PO_4?

Solution

Since each mole of Na_3PO_4 contains three moles of Na^+, the concentration of Na^+ is

$$[Na^+] = (1.76 \times 10^{-3}\ M) \times \frac{3\ mol\ Na^+}{mol\ Na_3PO_4} = 5.28 \times 10^{-3}\ M$$

and pNa is

$$pNa = -\log[Na^+] = -\log(5.28 \times 10^{-3}) = 2.277$$

Remember that a pNa of 2.777 has three, not four, significant figures; the 2 that appears in the one's place indicates the power of 10 when we write [Na⁺] as 0.528×10^{-2} M.

✔ Example 2.2.4

What is the $[H^+]$ in a solution that has a pH of 5.16?

Solution

The concentration of H^+ is

$$pH = -\log[H^+] = 5.16$$

$$\log[H^+] = -5.16$$

$$[H^+] = 10^{-5.16} = 6.9 \times 10^{-6}\ M$$

Recall that if $\log(X) = a$, then $X = 10^a$.

? Exercise 2.2.2

What are the values for pNa and pSO_4 if we dissolve 1.5 g Na_2SO_4 in a total solution volume of 500.0 mL?

Answer

The concentrations of Na^+ and SO_4^{2-} are

$$\frac{1.5\ g\ Na_2SO_4}{0.500L} \times \frac{1\ mol\ Na_2SO_4}{142.0\ g\ Na_2SO_4} \times \frac{2\ mol\ Na^+}{mol\ molNa_2SO_4} = 4.23 \times 10^{-2}\ M\ Na^+$$

$$\frac{1.5\ g\ Na_2SO_4}{0.500L} \times \frac{1\ mol\ Na_2SO_4}{142.0\ g\ Na_2SO_4} \times \frac{1\ mol\ SO_4^{2-}}{mol\ molNa_2SO_4} = 2.11 \times 10^{-2}\ M\ SO_4^{2-}$$

The pNa and pSO_4 values are

$$pNa = -\log(4.23 \times 10^{-2}\ M\ Na^+) = 1.37$$

$$pSO_4 = -\log(2.11 \times 10^{-2}\ M\ SO_4^{2-}) = 1.68$$

3: The Vocabulary of Analytical Chemistry

If you browse through an issue of the journal *Analytical Chemistry*, you will discover that the authors and readers share a common vocabulary of analytical terms. You probably are familiar with some of these terms, such as accuracy and precision, but other terms, such as analyte and matrix, are perhaps less familiar to you. In order to participate in any community, one must first understand its vocabulary; the goal of this chapter, therefore, is to introduce some important analytical terms. Becoming comfortable with these terms will make the chapters that follow easier to read and to understand.

3.1: Analysis, Determination, and Measurement

The first important distinction we will make is among the terms analysis, determination, and measurement. An analysis provides chemical or physical information about a sample. The component in the sample of interest to us is called the ***analyte***, and the remainder of the sample is the matrix. In an analysis we determine the identity, the concentration, or the properties of an analyte. To make this determination we measure one or more of the analyte's chemical or physical properties.

An example will help clarify the difference between an ***analysis***, a ***determination*** and a ***measurement***. In 1974 the federal government enacted the Safe Drinking Water Act to ensure the safety of the nation's public drinking water supplies. To comply with this act, municipalities monitor their drinking water supply for potentially harmful substances, such as fecal coliform bacteria. Municipal water departments collect and analyze samples from their water supply. To determine the concentration of fecal coliform bacteria an analyst passes a portion of water through a membrane filter, places the filter in a dish that contains a nutrient broth, and incubates the sample for 22–24 hrs at 44.5 ^{o}C ± 0.2 ^{o}C. At the end of the incubation period the analyst counts the number of bacterial colonies in the dish and reports the result as the number of colonies per 100 mL (Figure 3.1.1). Thus, a municipal water department analyzes samples of water to determine the concentration of fecal coliform bacteria by measuring the number of bacterial colonies that form during a carefully defined incubation period

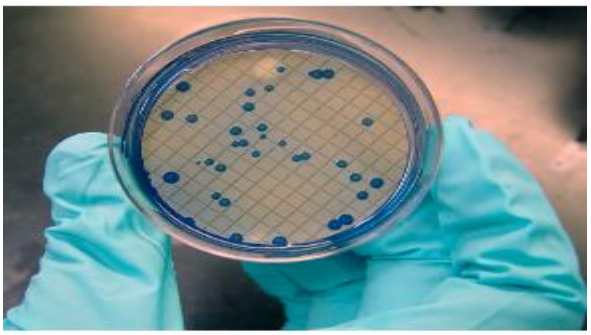


Figure 3.1.1 : Colonies of fecal coliform bacteria from a water supply. Source: Susan Boyer. Photo courtesy of ARS–USDA (www.ars.usda.gov).

> A fecal coliform count provides a general measure of the presence of pathogenic organisms in a water supply. For drinking water, the current maximum contaminant level (MCL) for total coliforms, including fecal coliforms is less than 1 colony/100 mL. Municipal water departments must regularly test the water supply and must take action if more than 5% of the samples in any month test positive for coliform bacteria.

3.2: Techniques, Methods, Procedures, and Protocols

Suppose you are asked to develop an analytical method to determine the concentration of lead in drinking water. How would you approach this problem? To provide a structure for answering this question, it is helpful to consider four levels of analytical methodology: techniques, methods, procedures, and protocols [Taylor, J. K. *Anal. Chem.* **1983**, *55*, 600A–608A].

A ***technique*** is any chemical or physical principle that we can use to study an analyte. There are many techniques for that we can use to determine the concentration of lead in drinking water [Fitch, A.; Wang, Y.; Mellican, S.; Macha, S. *Anal. Chem.* **1996**, *68*, 727A–731A]. In graphite furnace atomic absorption spectroscopy (GFAAS), for example, we first convert aqueous lead ions into free atoms—a process we call atomization. We then measure the amount of light absorbed by the free atoms. Thus, GFAAS uses both a chemical principle (atomization) and a physical principle (absorption of light).

A ***method*** is the application of a technique for a specific analyte in a specific matrix. As shown in Figure 3.2.1 , the GFAAS method for determining the concentration of lead in water is different from that for lead in soil or blood.

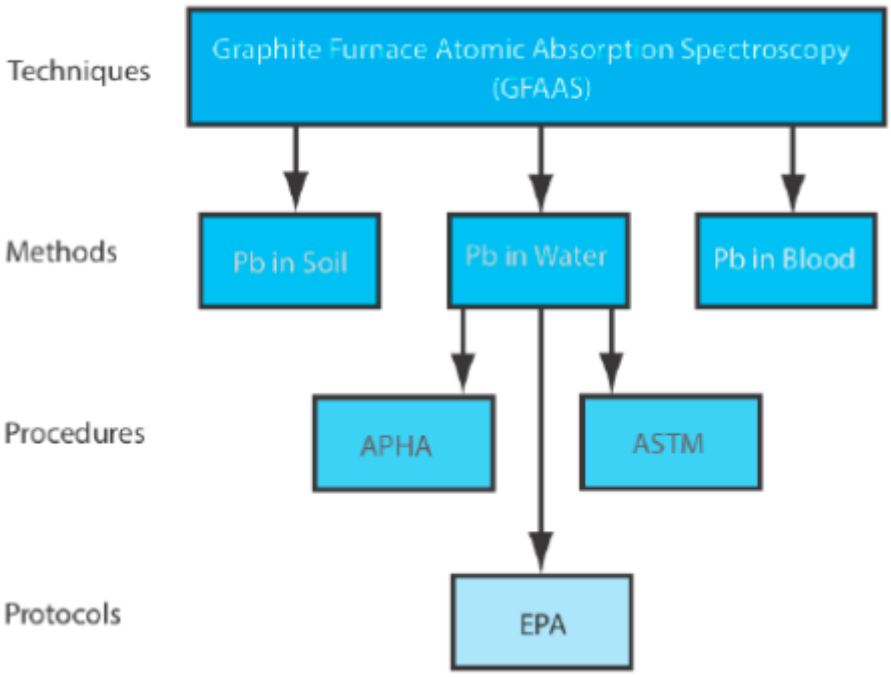


Figure 3.2.1 : Chart showing the hierarchical relationship between a technique, methods that use the technique, and procedures and protocols for a method. The abbreviations are APHA: American Public Health Association, ASTM: American Society for Testing Materials, EPA: Environmental Protection Agency.

A ***procedure*** is a set of written directions that tell us how to apply a method to a particular sample, including information on how to collect the sample, how to handle interferents, and how to validate results. A method may have several procedures as each analyst or agency adapts it to a specific need. As shown in Figure 3.2.1 , the American Public Health Agency and the American Society for Testing Materials publish separate procedures for determining the concentration of lead in water.

Finally, a ***protocol*** is a set of stringent guidelines that specify a procedure that an analyst must follow if an agency is to accept the results. Protocols are common when the result of an analysis supports or defines public policy. When determining the concentration of lead in water under the Safe Drinking Water Act, for example, the analyst must use a protocol specified by the Environmental Protection Agency.

There is an obvious order to these four levels of analytical methodology. Ideally, a protocol uses a previously validated procedure. Before developing and validating a procedure, a method of analysis must be selected. This requires, in turn, an initial screening of available techniques to determine those that have the potential for monitoring the analyte.

3.3: Classifying Analytical Techniques

The analysis of a sample generates a chemical or physical signal that is proportional to the amount of analyte in the sample. This signal may be anything we can measure, such as volume or absorbance. It is convenient to divide analytical techniques into two general classes based on whether the signal is proportional to the mass or moles of analyte, or is proportional to the analyte's concentration

Consider the two graduated cylinders in Figure 3.3.1 , each of which contains a solution of 0.010 M $Cu(NO_3)_2$. Cylinder 1 contains 10 mL, or $1.0\times10-41.0\times10-4$ moles of Cu^{2+}, and cylinder 2 contains 20 mL, or $2.0\times10-42.0\times10-4$ moles of Cu^{2+}. If a technique responds to the absolute amount of analyte in the sample, then the signal due to the analyte S_A

$$S_A = k_A n_A \qquad (3.3.1)$$

where n_A is the moles or grams of analyte in the sample, and k_A is a proportionality constant. Because cylinder 2 contains twice as many moles of Cu^{2+} as cylinder 1, analyzing the contents of cylinder 2 gives a signal twice as large as that for cylinder 1.

Figure 3.3.1 : Two graduated cylinders, each containing 0.10 M $Cu(NO_3)_2$. Although the cylinders contain the same concentration of Cu^{2+}, the cylinder on the left contains $1.0\times10-41.0\times10-4$ mol Cu^{2+} and the cylinder on the right contains $2.0\times10-42.0\times10-4$ mol Cu^{2+}.

A second class of analytical techniques are those that respond to the analyte's concentration, C_A

$$S_A = k_A C_A \quad (3.3.2)$$

Since the solutions in both cylinders have the same concentration of Cu^{2+}, their analysis yields identical signals.

A technique that responds to the absolute amount of analyte is a ***total analysis*** technique. Mass and volume are the most common signals for a total analysis technique, and the corresponding techniques are gravimetry (Chapter 8) and titrimetry (Chapter 9). With a few exceptions, the signal for a total analysis technique is the result of one or more chemical reactions, the stoichiometry of which determines the value of k_A in Equation 3.3.13.3.1.

Historically, most early analytical methods used a total analysis technique. For this reason, total analysis techniques are often called "classical" techniques.

Spectroscopy (Chapter 10) and electrochemistry (Chapter 11), in which an optical or an electrical signal is proportional to the relative amount of analyte in a sample, are examples of concentration techniques. The relationship between the signal and the analyte's concentration is a theoretical function that depends on experimental conditions and the instrumentation used to measure the signal. For this reason the value of k_A in Equation 3.3.23.3.2 is determined experimentally.

Since most concentration techniques rely on measuring an optical or electrical signal, they also are known as "instrumental" techniques.

3.4: Selecting an Analytical Method

A method is the application of a technique to a specific analyte in a specific matrix. We can develop an analytical method to determine the concentration of lead in drinking water using any of the techniques mentioned in the previous section. A gravimetric method, for example, might precipiate the lead as $PbSO_4$ or as $PbCrO_4$, and use the precipitate's mass as the analytical signal. Lead forms several soluble complexes, which we can use to design a complexation titrimetric method. As shown in Figure 3.2.1, we can use graphite furnace atomic absorption spectroscopy to determine the concentration of lead in drinking water. Finally, lead's multiple oxidation states (Pb^0, Pb^{2+}, Pb^{4+}) makes feasible a variety of electrochemical methods.

Ultimately, the requirements of the analysis determine the best method. In choosing among the available methods, we give consideration to some or all the following design criteria: accuracy, precision, sensitivity, selectivity, robustness, ruggedness, scale of operation, analysis time, availability of equipment, and cost.

Accuracy

Accuracy is how closely the result of an experiment agrees with the "true" or expected result. We can express accuracy as an absolute error, e

$$e = \text{obtained result} - \text{expected result}$$

or as a percentage relative error, $\%e_r$

$$\%e_r = \frac{\text{obtained result} - \text{expected result}}{\text{expected result}} \times 100$$

A method's accuracy depends on many things, including the signal's source, the value of k_A in Equation 3.3.1 or Equation 3.3.2, and the ease of handling samples without loss or contamination. A total analysis technique, such as gravimetry and titrimetry, often produce more accurate results than does a concentration technique because we can measure mass and volume with high accuracy, and because the value of k_A is known exactly through stoichiometry.

Because it is unlikely that we know the true result, we use an expected or accepted result to evaluate accuracy. For example, we might use a standard reference material, which has an accepted value, to establish an analytical method's accuracy. You will find a more detailed treatment of accuracy in Chapter 4, including a discussion of sources of errors.

Precision

When a sample is analyzed several times, the individual results vary from trial-to-trial. ***Precision*** is a measure of this variability. The closer the agreement between individual analyses, the more precise the results. For example, the results shown in the upper half of Figure 3.4.1 for the concentration of K^+ in a sample of serum are more precise than those in the lower half of Figure 3.4.1 . It is important to understand that precision does not imply accuracy. That the data in the upper half of Figure 3.4.1 are more precise does not mean that the first set of results is more accurate. In fact, neither set of results may be accurate.

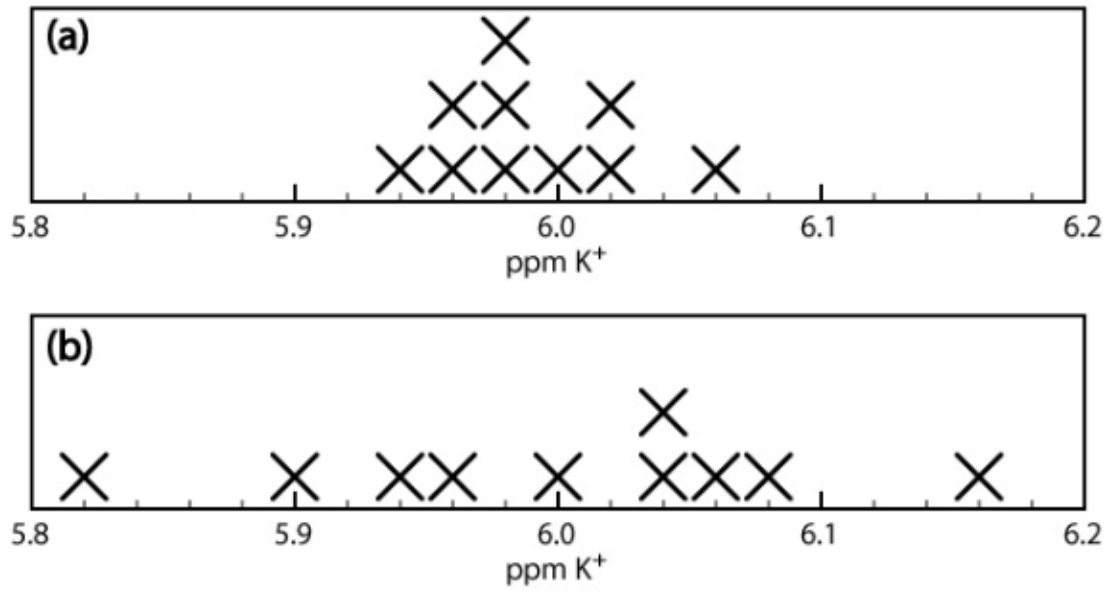


Figure 3.4.1 : Two determinations of the concentration of K^+ in serum, showing the effect of precision on the distribution of individual results. The data in (a) are less scattered and, therefore, more precise than the data in (b).

A method's precision depends on several factors, including the uncertainty in measuring the signal and the ease of handling samples reproducibly. In most cases we can measure the signal for a total analysis technique with a higher precision than is the case for a concentration method.

Confusing accuracy and precision is a common mistake. See Ryder, J.; Clark, A. *U. Chem. Ed.* **2002**, *6*, 1–3, and Tomlinson, J.; Dyson, P. J.; Garratt, J. *U. Chem. Ed.* **2001**, *5*, 16–23 for discussions of this and other common misconceptions about the meaning of error. You will find a more detailed treatment of precision in Chapter 4, including a discussion of sources of errors.

Sensitivity

The ability to demonstrate that two samples have different amounts of analyte is an essential part of many analyses. A method's ***sensitivity*** is a measure of its ability to establish that such a difference is significant. Sensitivity is often confused with a method's ***detection limit***, which is the smallest amount of analyte we can determine with confidence.

Confidence, as we will see in Chapter 4, is a statistical concept that builds on the idea of a population of results. For this reason, we will postpone our discussion of detection limits to Chapter 4. For now, the definition of a detection limit given here is sufficient.

Sensitivity is equivalent to the proportionality constant, k_A, in Equation 3.3.1 and Equation 3.3.2 [IUPAC Compendium of Chemical Terminology, Electronic version]. If ΔS_A is the smallest difference we can measure between two signals, then the smallest detectable difference in the absolute amount or the relative amount of analyte is

$$\Delta n_A = \frac{\Delta S_A}{k_A} \quad \text{or} \quad \Delta C_A = \frac{\Delta S_A}{k_A}$$

suppose, for example, that our analytical signal is a measurement of mass using a balance whose smallest detectable increment is ±0.0001 g. If our method's sensitivity is 0.200, then our method can conceivably detect a difference in mass of as little as

$$\Delta n_A = \frac{\pm 0.0001 \text{ g}}{0.200} = \pm 0.0005 \text{ g}$$

For two methods with the same ΔS_A, the method with the greater sensitivity—that is, the method with the larger k_A—is better able to discriminate between smaller amounts of analyte.

Specificity and Selectivity

An analytical method is specific if its signal depends only on the analyte [Persson, B-A; Vessman, J. *Trends Anal. Chem.* **1998**, *17*, 117–119; Persson, B-A; Vessman, J. *Trends*

Anal. Chem. **2001**, *20*, 526–532]. Although ***specificity*** is the ideal, few analytical methods are free from interferences. When an ***interferent*** contributes to the signal, we expand Equation 3.3.1 and Equation 3.3.2 to include its contribution to the sample's signal, S_{samp}

$$S_{samp} = S_A + S_I = k_A n_A + k_I n_I$$

$$S_{samp} = S_A + S_I = k_A C_A + k_I C_I$$

where S_I is the interferent's contribution to the signal, k_I is the interferent's sensitivity, and n_I and C_I are the moles (or grams) and the concentration of interferent in the sample, respectively.

Selectivity is a measure of a method's freedom from interferences [Valcárcel, M.; Gomez-Hens, A.; Rubio, S. *Trends Anal. Chem.* **2001**, *20*, 386–393]. A method's selectivity for an interferent relative to the analyte is defined by a ***selectivity coefficient***, $K_{A,I}$

$$K_{A,I} = \frac{k_I}{k_A} \qquad (3.4.3)$$

which may be positive or negative depending on the signs of k_I and k_A. The selectivity coefficient is greater than +1 or less than –1 when the method is more selective for the interferent than for the analyte.

Although k_A and k_I usually are positive, they can be negative. For example, some analytical methods work by measuring the concentration of a species that remains after is reacts with the analyte. As the analyte's concentration increases, the concentration of the species that produces the signal decreases, and the signal becomes smaller. If the signal in the absence of analyte is assigned a value of zero, then the subsequent signals are negative.

Determining the selectivity coefficient's value is easy if we already know the values for k_A and k_I. As shown by Example 3.4.1 , we also can determine $K_{A,I}$ by measuring S_{samp} in the presence of and in the absence of the interferent.

✔ Example 3.4.1

A method for the analysis of Ca^{2+} in water suffers from an interference in the presence of Zn^{2+}. When the concentration of Ca^{2+} is 100 times greater than that of Zn^{2+}, an analysis for Ca^{2+} has a relative error of +0.5%. What is the selectivity coefficient for this method?

Solution

Since only relative concentrations are reported, we can arbitrarily assign absolute concentrations. To make the calculations easy, we will let C_{Ca} = 100 (arbitrary units) and C_{Zn} = 1. A relative error of +0.5% means the signal in the presence of Zn^{2+} is 0.5% greater than the signal in the absence of Zn^{2+}. Again, we can assign values to make the calculation easier. If the signal for Cu^{2+} in the absence of Zn^{2+} is 100 (arbitrary units), then the signal in the presence of Zn^{2+} is 100.5.

The value of k_{Ca} is determined using Equation 3.3.2

$$k_{Ca} = \frac{S_{Ca}}{C_{Ca}} = \frac{100}{100} = 1$$

In the presence of Zn^{2+} the signal is given by Equation 3.4.2; thus

$$S_{samp} = 100.5 = k_{Ca} C_{Ca} + k_{Zn} C_{Zn} = (1 \times 100) + k_{Zn} \times 1$$

Solving for k_{Zn} gives its value as 0.5. The selectivity coefficient is

$$K_{Ca,Zn} = \frac{k_{Zn}}{k_{Ca}} = \frac{0.5}{1} = 0.5$$

If you are unsure why, in the above example, the signal in the presence of zinc is 100.5, note that the percentage relative error for this problem is given by

$$\frac{\text{obtained result} - 100}{100} \times 100 = +0.5\%$$

Solving gives an obtained result of 100.5.

? Exercise 3.4.1

Wang and colleagues describe a fluorescence method for the analysis of Ag^+ in water. When analyzing a solution that contains 1.0×10^{-9} M Ag^+ and 1.1×10^{-7} M Ni^{2+}, the fluorescence intensity (the signal) was +4.9% greater than that obtained for a sample of 1.0×10^{-9} M Ag^+. What is $K_{Ag,Ni}$ for this analytical method? The full citation for the data in this exercise is Wang, L.; Liang, A. N.; Chen, H.; Liu, Y.; Qian, B.; Fu, J. *Anal. Chim. Acta* **2008**, *616*, 170-176.

Answer

A selectivity coefficient provides us with a useful way to evaluate an interferent's potential effect on an analysis. Solving Equation 3.4.3 for k_I

$$k_I = K_{A,I} \times k_A \tag{3.4.4}$$

and substituting in Equation 3.4.1 and Equation 3.4.2, and simplifying gives

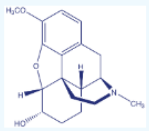
$$S_{samp} = k_A\{n_A + K_{A,I} \times n_I\} \tag{3.4.5}$$

$$S_{samp} = k_A\{C_A + K_{A,I} \times C_I\} \tag{3.4.6}$$

An interferent will not pose a problem as long as the term $K_{A,I} \times n_I$ in Equation 3.4.5 is significantly smaller than n_A, or if $K_{A,I} \times C_I$ in Equation 3.4.6 is significantly smaller than C_A.

✔ Example 3.4.2

Barnett and colleagues developed a method to determine the concentration of codeine (structure shown below) in poppy plants [Barnett, N. W.; Bowser, T. A.; Geraldi, R. D.; Smith, B. *Anal. Chim. Acta* **1996**, *318*, 309– 317]. As part of their study they evaluated the effect of several interferents. For example, the authors found that equimolar solutions of codeine and the interferent 6-methoxycodeine gave signals, respectively of 40 and 6 (arbitrary units).



(a) What is the selectivity coefficient for the interferent, 6-methoxycodeine, relative to that for the analyte, codeine.

(b) If we need to know the concentration of codeine with an accuracy of ±0.50%, what is the maximum relative concentration of 6-methoxy-codeine that we can tolerate?

Solution

(a) The signals due to the analyte, S_A, and the interferent, S_I, are

$$S_A = k_A C_A \qquad S_I = k_I C_I$$

Solving these equations for k_A and for k_I, and substituting into Equation 3.4.4 gives

$$K_{A,I} = \frac{S_I / C_I}{S_A / C_I}$$

Because the concentrations of analyte and interferent are equimolar ($C_A = C_I$), the selectivity coefficient is

$$K_{A,I} = \frac{S_I}{S_A} = \frac{6}{40} = 0.15$$

(b) To achieve an accuracy of better than ±0.50% the term $K_{A,I} \times C_I$ in Equation 3.4.6 must be less than 0.50% of C_A; thus

$$K_{A,I} \times C_I \leq 0.0050 \times C_A$$

Solving this inequality for the ratio C_I/C_A and substituting in the value for $K_{A,I}$ from part (a) gives

$$\frac{C_I}{C_A} \leq \frac{0.0050}{K_{A,I}} = \frac{0.0050}{0.15} = 0.033$$

Therefore, the concentration of 6-methoxycodeine must be less than 3.3% of codeine's concentration.

When a method's signal is the result of a chemical reaction—for example, when the signal is the mass of a precipitate—there is a good chance that the method is not very selective and that it is susceptible to an interference.

? Exercise 3.4.2

Mercury (II) also is an interferent in the fluorescence method for Ag^+ developed by Wang and colleagues (see Practice Exercise 3.4.1). The selectivity coefficient, $K_{Ag,Hg}$ has a value of -1.0×10^{-3}.

(a) What is the significance of the selectivity coefficient's negative sign?

(b) Suppose you plan to use this method to analyze solutions with concentrations of Ag^+ no smaller than 1.0 nM. What is the maximum concentration of Hg^{2+} you can tolerate if your percentage relative errors must be less than ±1.0%?

Answer

(a) A negative value for $K_{Ag,Hg}$ means that the presence of Hg^{2+} decreases the signal from Ag^+.

(b) In this case we need to consider an error of −1%, since the effect of Hg^{2+} is to decrease the signal from Ag^+. To achieve this error, the term $K_{A,I} \times C_I$ in Equation 3.4.6 must be less than −1% of C_A; thus

$$K_{Ag,Hg} \times C_{Hg} = -0.01 \times C_{Ag}$$

Substituting in known values for $K_{Ag,Hg}$ and C_{Ag}, we find that the maximum concentration of Hg^{2+} is 1.0×10^{-8} M.

Problems with selectivity also are more likely when the analyte is present at a very low concentration [Rodgers, L. B. *J. Chem. Educ.* **1986**, *63*, 3–6].

Look back at Figure 1.1.1, which shows Fresenius' analytical method for the determination of nickel in ores. The reason there are so many steps in this procedure is that precipitation reactions generally are not very selective. The method in Figure 1.1.2 includes fewer steps because dimethylglyoxime is a more selective reagent. Even so, if an ore contains palladium, additional steps are needed to prevent the palladium from interfering.

Robustness and Ruggedness

For a method to be useful it must provide reliable results. Unfortunately, methods are subject to a variety of chemical and physical interferences that contribute uncertainty to the analysis. If a method is relatively free from chemical interferences, we can use it to analyze an analyte in a wide variety of sample matrices. Such methods are considered ***robust***.

Random variations in experimental conditions introduces uncertainty. If a method's sensitivity, *k*, is too dependent on experimental conditions, such as temperature, acidity, or reaction time, then a slight change in any of these conditions may give a significantly different result. A ***rugged*** method is relatively insensitive to changes in experimental conditions.

Scale of Operation

Another way to narrow the choice of methods is to consider three potential limitations: the amount of sample available for the analysis, the expected concentration of analyte in the samples, and the minimum amount of analyte that will produce a measurable signal. Collectively, these limitations define the analytical method's scale of operations.

We can display the scale of operations visually (Figure 3.4.2) by plotting the sample's size on the *x*-axis and the analyte's concentration on the *y*-axis. For convenience, we divide samples into macro (>0.1 g), meso (10 mg–100 mg), micro (0.1 mg–10 mg), and ultramicro (<0.1 mg) sizes, and we divide analytes into major (>1% w/w), minor (0.01% w/w–1% w/w), trace (10^{-7}% w/w–0.01% w/w), and ultratrace (<10^{-7}% w/w) components. Together, the analyte's concentration and the sample's size provide a characteristic description for an analysis. For example, in a microtrace analysis the sample weighs between 0.1

mg and 10 mg and contains a concentration of analyte between 10^{-7}% w/w and 10^{-2}% w/w.

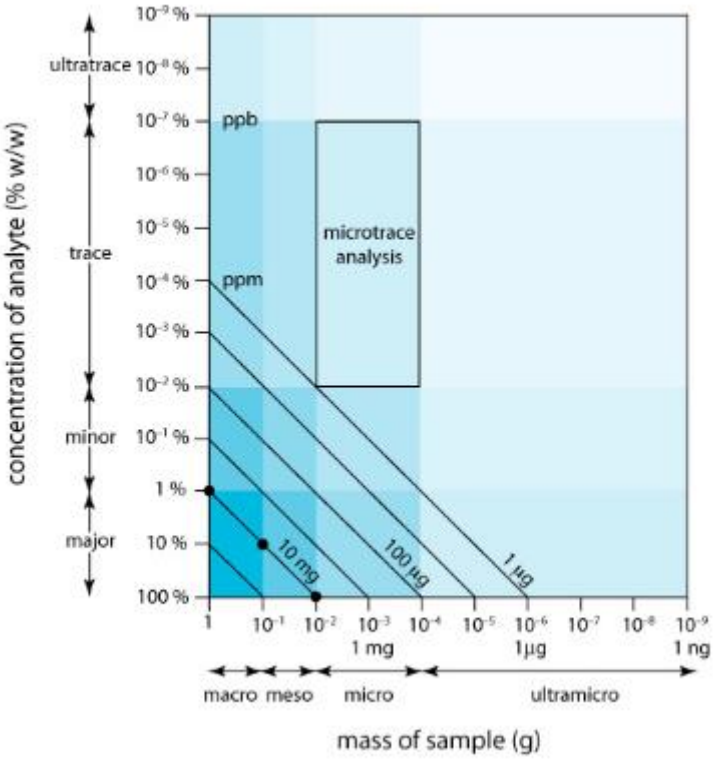


Figure 3.4.2 : Scale of operations for analytical methods. The shaded areas define different types of analyses. The boxed area, for example, represents a microtrace analysis. The diagonal lines show combinations of sample size and analyte concentration that contain the same mass of analyte. The three filled circles (•), for example, indicate analyses that use 10 mg of analyte. See Sandell, E. B.; Elving, P. J. in Kolthoff, I. M.; Elving, P. J., eds. *Treatise on Analytical Chem-istry*, Interscience: New York, Part I, Vol. 1, Chapter 1, pp. 3–6; (b) Potts, L. W. *Quantitative Analysis–Theory and Practice*, Harper and Row: New York, 1987, pp. 12 for more details.

The diagonal lines connecting the axes show combinations of sample size and analyte concentration that contain the same absolute mass of analyte. As shown in Figure 3.4.2 , for example, a 1-g sample that is 1% w/w analyte has the same amount of analyte (10 mg) as a 100-mg sample that is 10% w/w analyte, or a 10-mg sample that is 100% w/w analyte.

We can use Figure 3.4.2 to establish limits for analytical methods. If a method's minimum detectable signal is equivalent to 10 mg of analyte, then it is best suited to a major analyte in a macro or meso sample. Extending the method to an analyte with a concentration of 0.1% w/w requires a sample of 10 g, which rarely is practical due to the complications of carrying such a large amount of material through the analysis. On the other hand, a small sample that contains a trace amount of analyte places significant restrictions on an analysis. For example, a 1-mg sample that is 10^{-4}% w/w in analyte contains just 1 ng of analyte. If we isolate the analyte in 1 mL of solution, then we need an analytical method that reliably can detect it at a concentration of 1 ng/mL.

It should not surprise you to learn that a total analysis technique typically requires a macro or a meso sample that contains a major analyte. A concentration

technique is particularly useful for a minor, trace, or ultratrace analyte in a macro, meso, or micro sample.

Equipment, Time, and Cost

Finally, we can compare analytical methods with respect to their equipment needs, the time needed to complete an analysis, and the cost per sample. Methods that rely on instrumentation are equipment-intensive and may require significant operator training. For example, the graphite furnace atomic absorption spectroscopic method for determining lead in water requires a significant capital investment in the instrument and an experienced operator to obtain reliable results. Other methods, such as titrimetry, require less expensive equipment and less training.

The time to complete an analysis for one sample often is fairly similar from method-to-method. This is somewhat misleading, however, because much of this time is spent preparing samples, preparing reagents, and gathering together equipment. Once the samples, reagents, and equipment are in place, the sampling rate may differ substantially. For example, it takes just a few minutes to analyze a single sample for lead using graphite furnace atomic absorption spectroscopy, but several hours to analyze the same sample using gravimetry. This is a significant factor in selecting a method for a laboratory that handles a high volume of samples.

The cost of an analysis depends on many factors, including the cost of equipment and reagents, the cost of hiring analysts, and the number of samples that can be processed per hour. In general, methods that rely on instruments cost more per sample then other methods.

Making the Final Choice

Unfortunately, the design criteria discussed in this section are not mutually independent [Valcárcel, M.; Ríos, A. *Anal. Chem.* **1993**, *65*, 781A–787A]. Working with smaller samples or improving selectivity often comes at the expense of precision. Minimizing cost and analysis time may decrease accuracy. Selecting a method requires carefully balancing the various design criteria. Usually, the most important design criterion is accuracy, and the best method is the one that gives the most accurate result. When the need for a result is urgent, as is often the case in clinical labs, analysis time may become the critical factor.

In some cases it is the sample's properties that determine the best method. A sample with a complex matrix, for example, may require a method with excellent selectivity to avoid interferences. Samples in which the analyte is present at a trace or ultratrace concentration usually require a concentration method. If the quantity of sample is limited, then the method must not require a large amount of sample.

Determining the concentration of lead in drinking water requires a method that can detect lead at the parts per billion concentration level. Selectivity is important because other metal ions are present at significantly higher concentrations. A method that uses graphite furnace atomic absorption spectroscopy is a common choice for determining lead in drinking water because it meets these specifications. The same method is also useful for determining lead in blood where its ability to detect low concentrations of lead using a few microliters of sample is an important consideration.

3.5: Developing the Procedure

After selecting a method, the next step is to develop a procedure that accomplish our goals for the analysis. In developing a procedure we give attention to compensating for interferences, to selecting and calibrating equipment, to acquiring a representative sample, and to validating the method.

Compensating for Interferences

A method's accuracy depends on its selectivity for the analyte. Even the best method, however, may not be free from interferents that contribute to the measured signal. Potential interferents may be present in the sample itself or in the reagents used during the analysis.

When the sample is free of interferents, the total signal, S_{total}, is a sum of the signal due to the analyte, S_A, and the signal due to interferents in the reagents, S_{reag},

$$S_{total} = S_A + S_{reag} = k_A n_A + S_{reag} \tag{3.5.1}$$

$$S_{total} = S_A + S_{reag} = k_A C_A + S_{reag} \tag{3.5.2}$$

Without an independent determination of S_{reag} we cannot solve Equation 3.5.1 or 3.5.2 for the moles or concentration of analyte.

To determine the contribution of S_{reag} in Equations 3.5.1 and 3.5.2 we measure the signal for a ***method blank***, a solution that does not contain the sample. Consider, for example, a procedure in which we dissolve a 0.1-g sample in a portion of solvent, add several reagents, and dilute to 100 mL with additional solvent. To prepare the method blank we omit the sample and dilute the reagents to 100 mL using the solvent. Because the analyte is absent, S_{total} for the method blank is equal to S_{reag}. Knowing the value for S_{reag} makes it is easy to correct S_{total} for the reagent's contribution to the total signal; thus

$$(S_{total} - S_{reag}) = S_A = k_A n_A$$

$$(S_{total} - S_{reag}) = S_A = k_A C_A$$

By itself, a method blank cannot compensate for an interferent that is part of the sample's matrix. If we happen to know the interferent's identity and concentration, then we can be add it to the method blank; however, this is not a common circumstance and we must, instead, find a method for separating the analyte and interferent before continuing the analysis.

A method blank also is known as a reagent blank. When the sample is a liquid, or is in solution, we use an equivalent volume of an inert solvent as a substitute for the sample.

Calibration

A simple definition of a quantitative analytical method is that it is a mechanism for converting a measurement, the signal, into the amount of analyte in a sample. Assuming we can correct for interferents, a quantitative analysis is nothing more than solving Equation 3.3.1 or Equation 3.3.2 for n_A or for C_A.

To solve these equations we need the value of k_A. For a total analysis method usually we know the value of k_A because it is defined by the stoichiometry of the chemical reactions responsible for the signal. For a concentration method, however, the value of k_A usually is a complex function of experimental conditions. A ***calibration*** is the process of experimentally determining the value of k_A by measuring the signal for one or more standard samples, each of which contains a known concentration of analyte.

With a single standard we can calculate the value of k_A using Equation 3.3.1 or Equation 3.3.2. When using several standards with different concentrations of analyte, the result is best viewed visually by plotting S_A versus the concentration of analyte in the standards. Such a plot is known as a ***calibration curve***, an example of which is shown in Figure 3.5.1

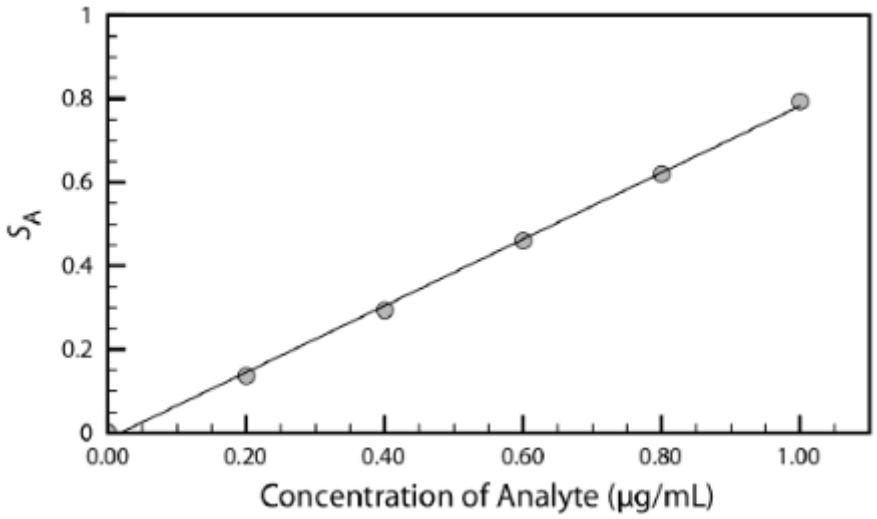


Figure 3.5.1 : Example of a calibration curve. The filled circles (•) are the results for five standard samples, each with a different concentration of analyte, and the line is the best fit to the data determined by a linear regression analysis. See Chapter 5 for a further discussion of calibration curves and an explanation of linear regression.

Sampling

Selecting an appropriate method and executing it properly helps us ensure that our analysis is accurate. If we analyze the wrong sample, however, then the accuracy of our work is of little consequence.

A proper sampling strategy ensures that our samples are representative of the material from which they are taken. Biased or nonrepresentative sampling, and contaminating samples during or after their collection are two examples of sampling errors that can lead to a significant error in accuracy. It is important to realize that sampling errors are independent of errors in the analytical method. As a result, we cannot correct a sampling error in the laboratory by, for example, evaluating a reagent blank.

Chapter 7 provides a more detailed discussion of sampling, including strategies for obtaining representative samples.

Validation

If we are to have confidence in our procedure we must demonstrate that it can provide acceptable results, a process we call ***validation***. Perhaps the most important part of validating a procedure is establishing that its precision and accuracy are appropriate for the problem we are trying to solve. We also ensure that the written procedure has sufficient detail so that different analysts or laboratories will obtain comparable results. Ideally, validation uses a standard sample whose composition closely matches the samples we will analyze. In the absence of appropriate standards, we can evaluate accuracy by comparing results to those obtained using a method of known accuracy.

3.6: Protocols

Earlier we defined a protocol as a set of stringent written guidelines that specify an exact procedure that we must follow if an agency is to accept the results of our analysis. In addition to the considerations that went into the procedure's design, a protocol also contains explicit instructions regarding internal and external quality assurance and quality control (***QA/QC***) procedures [Amore, F. *Anal. Chem.* **1979**, *51*, 1105A–1110A; Taylor, J. K. *Anal. Chem.* **1981**, *53*, 1588A–1593A]. The goal of internal QA/QC is to ensure that a laboratory's work is both accurate and precise. External QA/QC is a process in which an external agency certifies a laboratory.

As an example, let's outline a portion of the Environmental Protection Agency's protocol for determining trace metals in water by graphite furnace atomic absorption spectroscopy as part of its Contract Laboratory Program (CLP). The CLP protocol (see Figure 3.6.1) calls for an initial calibration using a method blank and three standards, one of which is at the detection limit. The resulting calibration curve is verified by analyzing initial calibration verification (ICV) and initial calibration blank (ICB) samples. The lab's result for the ICV sample must fall within ±10% of its expected concentration. If the result is outside this limit the analysis is stopped and the problem identified and corrected before continuing.

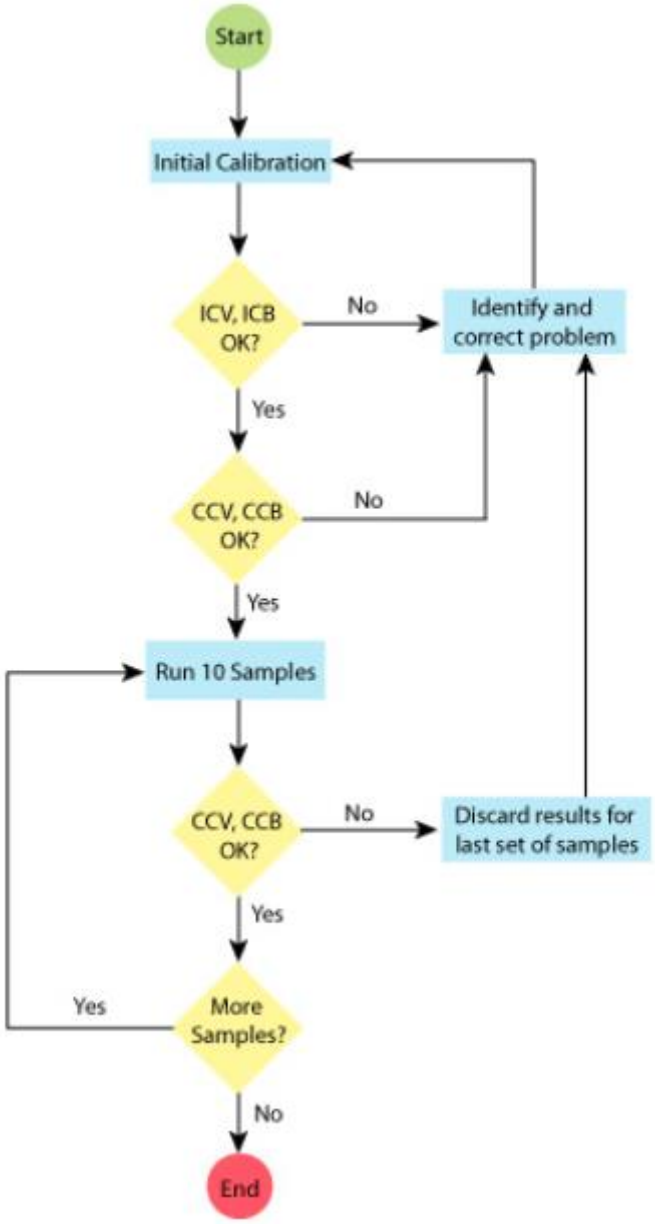


Figure 3.6.1 : Schematic diagram showing a portion of the EPA's protocol for determining trace metals in water using graphite furnace atomic absorption spectrometry. The abbreviations are ICV: initial calibration verification; ICB: initial calibration blank; CCV: continuing calibration verification; CCB: continuing calibration blank.

After a successful analysis of the ICV and ICB samples, the lab reverifies the calibration by analyzing a continuing calibration verification (CCV) sample and a continuing calibration blank (CCB). Results for the CCV also must be within ±10% of its expected concentration. Again, if the lab's result for the CCV is outside the established limits, the analysis is stopped, the problem identified and corrected, and the system recalibrated as described above. Additional CCV and the CCB samples are analyzed before the first sample and after the last sample, and between every set of ten samples. If the result for any CCV or CCB sample is unacceptable, the results for the last set of samples are discarded, the system is recalibrated, and the samples reanalyzed. By following this protocol, each result is bound

by successful checks on the calibration. Although not shown in Figure 3.6.1, the protocol also contains instructions for analyzing duplicate or split samples, and for using spike tests to verify accuracy.

3.7: The Importance of Analytical Methodology

The importance of the issues raised in this chapter is evident if we examine environmental monitoring programs. The purpose of a monitoring program is to determine the present status of an environmental system, and to assess long term trends in the system's health. These are broad and poorly defined goals. In many cases, an environmental monitoring program begins before the essential questions are known. This is not surprising since it is difficult to formulate questions in the absence of results. Without careful planning, however, a poor experimental design may result in data that has little value.

These concerns are illustrated by the Chesapeake Bay Monitoring Program. This research program, designed to study nutrients and toxic pollutants in the Chesapeake Bay, was initiated in 1984 as a cooperative venture between the federal government, the state governments of Maryland, Virginia, and Pennsylvania, and the District of Columbia. A 1989 review of the program highlights the problems common to many monitoring programs [D'Elia, C. F.; Sanders, J. G.; Capone, D. G. *Envrion. Sci. Technol.* **1989**, *23*, 768–774].

At the beginning of the Chesapeake Bay monitoring program, little attention was given to selecting analytical methods, in large part because the eventual use of the data was not yet specified. The analytical methods initially chosen were standard methods already approved by the Environmental Protection Agency (EPA). In many cases these methods were not useful because they were designed to detect pollutants at their legally mandated maximum allowed concentrations. In unpolluted waters, however, the concentrations of these contaminants often are well below the detection limit of the EPA methods. For example, the detection limit for the EPA approved standard method for phosphate was 7.5 ppb. Since the actual phosphate concentrations in Chesapeake Bay were below the EPA method's detection limit, it provided no useful information. On the other hand, the detection limit for a non-approved variant of the EPA method, a method routinely used by chemical oceanographers, was 0.06 ppb, a more realistic detection limit for their samples. In other cases, such as the elemental analysis for particulate forms of carbon, nitrogen and phosphorous, EPA approved procedures provided poorer reproducibility than nonapproved methods.

3.8: Problems

1. When working with a solid sample, often it is necessary to bring the analyte into solution by digesting the sample with a suitable solvent. Any remaining solid impurities are removed by filtration before continuing with the analysis. In a typical total analysis method, the procedure might read

   "After digesting the sample in a beaker using approximately 25 mL of

solvent, remove any solid impurities that remain by passing the solution the analyte through filter paper, collecting the filtrate in a clean Erlenmeyer flask. Rinse the beaker with several small portions of solvent, passing these rinsings through the filter paper and collecting them in the same Erlenmeyer flask. Finally, rinse the filter paper with several portions of solvent, collecting the rinsings in the same Erlenmeyer flask."

For a typical concentration method, however, the procedure might state

"After digesting the sample in a beaker using 25.00 mL of solvent, remove any solid impurities by filtering a portion of the solution containing the analyte. Collect and discard the first several mL of filtrate before collecting a sample of 5.00 mL for further analysis."

Explain why these two procedures are different.

2. A certain concentration method works best when the analyte's concentration is approximately 10 ppb.

   (a) If the method requires a sample of 0.5 mL, about what mass of analyte is being measured?

   (b) If the analyte is present at 10% w/v, how would you prepare the sample for analysis?

   (c) Repeat for the case where the analyte is present at 10% w/w.

   (d) Based on your answers to parts (a)–(c), comment on the method's suitability for the determination of a major analyte.

3. An analyst needs to evaluate the potential effect of an interferent, I, on the quantitative analysis for an analyte, A. She begins by measuring the signal for a sample in which the interferent is absent and the analyte is present with a concentration of 15 ppm, obtaining an average signal of 23.3 (arbitrary units). When she analyzes a sample in which the analyte is absent and the interferent is present with a concentration of 25 ppm, she obtains an average signal of 13.7.

   (a) What is the sensitivity for the analyte?

   (b) What is the sensitivity for the interferent?

   (c) What is the value of the selectivity coefficient?

   (d) Is the method more selective for the analyte or the interferent?

   (e) What is the maximum concentration of interferent relative to that of the analyte if the error in the analysis is to be less than 1%?

4. A sample is analyzed to determine the concentration of an analyte. Under the conditions of the analysis the sensitivity is $17.2\ \text{ppm}^{-1}$. What is the analyte's concentration if S_{total} is 35.2 and S_{reag} is 0.6?
5. A method for the analysis of Ca^{2+} in water suffers from an interference in the presence of Zn^{2+}. When the concentration of Ca^{2+} is 50 times greater than that of Zn^{2+}, an analysis for Ca^{2+} gives a relative error of –2.0%. What is the value of the selectivity coefficient for this method?
6. The quantitative analysis for reduced glutathione in blood is complicated by many potential interferents. In one study, when analyzing a solution of 10.0 ppb glutathione and 1.5 ppb ascorbic acid, the signal was 5.43 times greater than that obtained for the analysis of 10.0 ppb glutathione [Jiménez-Prieto, R.; Velasco, A.; Silva, M; Pérez-Bendito, D. *Anal. Chem. Acta* **1992**, *269*, 273– 279]. What is the selectivity coefficient for this analysis? The same study found that analyzing a solution of 3.5×10^{2} ppb methionine and 10.0 ppb glutathione gives a signal that is 0.906 times less than that obtained for the analysis of 10.0 ppb glutathione. What is the selectivity coefficient for this analysis? In what ways do these interferents behave differently?
7. Oungpipat and Alexander described a method for determining the concentration of glycolic acid (GA) in a variety of samples, including physiological fluids such as urine [Oungpipat, W.; Alexander, P. W. *Anal. Chim. Acta* **1994**, *295*, 36–46]. In the presence of only GA, the signal is

$$S_{samp,1} = k_{\text{GA}} C_{\text{GA}}$$

and in the presence of both glycolic acid and ascorbic acid (AA), the signal is

$$S_{samp,2} = k_{\text{GA}} C_{\text{GA}} + k_{\text{AA}} C_{\text{AA}}$$

When the concentration of glycolic acid is $1.0 \times 10^{-4}\ \text{M}$ and the concentration of ascorbic acid is $1.0 \times 10^{-5}\ \text{M}$, the ratio of their signals is

$$\frac{S_{samp,2}}{S_{samp,1}} = 1.44$$

1. (a) Using the ratio of the two signals, determine the value of the selectivity ratio $K_{\text{GA,AA}}$.

   (b) Is the method more selective toward glycolic acid or ascorbic acid?

   (c) If the concentration of ascorbic acid is $1.0\times10^{-5}\ \text{M}$, what is the smallest concentration of glycolic acid that can be determined such that the error introduced by failing to account for the signal from ascorbic acid is less than 1%?
2. Ibrahim and co-workers developed a new method for the quantitative analysis of hypoxanthine, a natural compound of some nucleic acids [Ibrahim, M. S.; Ahmad, M. E.; Temerk, Y. M.; Kaucke, A. M. *Anal. Chim. Acta* **1996**, *328*, 47–52]. As part of their study they evaluated the method's selectivity for hypoxanthine in the presence of several possible

interferents, including ascorbic acid.

(a) When analyzing a solution of 1.12×10^{-6} M hypoxanthine the authors obtained a signal of 7.45×10^{-5} amps. What is the sensitivity for hypoxanthine? You may assume the signal has been corrected for the method blank.

(b) When a solution containing 1.12×10^{-6} M hypoxanthine and 6.5×10^{-5} M ascorbic acid is analyzed a signal of 4.04×10^{-5} amps is obtained. What is the selectivity coefficient for this method?

(c) Is the method more selective for hypoxanthine or for ascorbic acid?

(d) What is the largest concentration of ascorbic acid that may be present if a concentration of 1.12×10^{-6} M hypoxanthine is to be determined within 1.0%?

3. Examine a procedure from *Standard Methods for the Analysis of Waters and Wastewaters* (or another manual of standard analytical methods) and identify the steps taken to compensate for interferences, to calibrate equipment and instruments, to standardize the method, and to acquire a representative sample.

4: Evaluating Analytical Data

When we use an analytical method we make three separate evaluations of experimental error. First, before we begin the analysis we evaluate potential sources of errors to ensure they will not adversely effect our results. Second, during the analysis we monitor our measurements to ensure that errors remain acceptable. Finally, at the end of the analysis we evaluate the quality of the measurements and results, and compare them to our original design criteria. This chapter provides an introduction to sources of error, to evaluating errors in analytical measurements, and to the statistical analysis of data.

4.1: Characterizing Measurements and Results

Let's begin by choosing a simple quantitative problem that requires a single measurement: What is the mass of a penny? You probably recognize that our statement of the problem is too broad. For example, are we interested in the mass of a United States penny or of a Canadian penny, or is the difference relevant? Because a penny's composition and size may differ from country to country, let's narrow our problem to pennies from the United States.

There are other concerns we might consider. For example, the United States Mint produces pennies at two locations (Figure 4.1.1). Because it seems unlikely that a penny's mass depends on where it is minted, we will ignore this concern. Another concern is whether the mass of a newly minted penny is different from the mass of a circulating penny. Because the answer this time is not obvious,

let's further narrow our question and ask "What is the mass of a circulating United States Penny?"

Figure 4.1.1 : An uncirculated 2005 Lincoln head penny. The "D" below the date indicates that this penny was produced at the United States Mint at Denver, Colorado. Pennies produced at the Philadelphia Mint do not have a letter below the date. Source: United States Mint image.

A good way to begin our analysis is to gather some preliminary data. Table 4.1.1 shows masses for seven pennies collected from my change jar. In examining this data we see that our question does not have a simple answer. That is, we can not use the mass of a single penny to draw a specific conclusion about the mass of any other penny (although we might reasonably conclude that all pennies weigh at least 3 g). We can, however, characterize this data by reporting the spread of the individual measurements around a central value.

Table 4.1.1 : Masses of Seven Circulating U. S. Pennies

Penny	Mass (g)
1	3.080
2	3.094
3	3.107
4	3.056
5	3.112
6	3.174
7	3.198

Measures of Central Tendency

One way to characterize the data in Table 4.1.1 is to assume that the masses of individual pennies are scattered randomly around a central value that is the best estimate of a penny's expected, or "true" mass. There are two common ways to estimate central tendency: the mean and the median.

Mean

The ***mean***, $\overline{X}$, is the numerical average for a data set. We calculate the mean by dividing the sum of the individual values by the size of the data set

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

where X_i is the i^{th} measurement, and n is the size of the data set.

✔ Example 4.1.1

What is the mean for the data in Table 4.1.1 ?

Solution

To calculate the mean we add together the results for all measurements

$$3.080 + 3.094 + 3.107 + 3.056 + 3.112 + 3.174 + 3.198 = 21.821 \text{ g}$$

and divide by the number of measurements

$$\overline{X} = \frac{21.821 \text{ g}}{7} = 3.117 \text{ g}$$

The mean is the most common estimate of central tendency. It is not a robust estimate, however, because a single extreme value—one much larger or much smaller than the remainder of the data—influences strongly the mean's value [Rousseeuw, P. J. *J. Chemom.* **1991**, *5*, 1–20]. For example, if we accidently record the third penny's mass as 31.07 g instead of 3.107 g, the mean changes from 3.117 g to 7.112 g!

An estimate for a statistical parameter is robust if its value is not affected too much by an unusually large or an unusually small measurement.

Median

The ***median***, $\widetilde{X}$, is the middle value when we order our data from the smallest to the largest value. When the data has an odd number of values, the median is the middle value. For an even number of values, the median is the average of the $n/2$ and the $(n/2) + 1$ values, where n is the size of the data set.

When $n = 5$, the median is the third value in the ordered data set; for $n = 6$, the median is the average of the third and fourth members of the ordered data set.

✔ Example 4.1.2

What is the median for the data in Table 4.1.1 ?

Solution

To determine the median we order the measurements from the smallest to the largest value

$$3.056 \quad 3.080 \quad 3.094 \quad 3.107 \quad 3.112 \quad 3.174 \quad 3.198$$

Because there are seven measurements, the median is the fourth value in the ordered data; thus, the median is 3.107 g.

As shown by Example 4.1.1 and Example 4.1.2 , the mean and the median provide similar estimates of central tendency when all measurements are comparable in magnitude. The median, however, is a more robust estimate of central tendency because it is less sensitive to measurements with extreme values. For example, if we accidently record the third penny's mass as 31.07 g instead of 3.107 g, the median's value changes from 3.107 g to 3.112 g.

Measures of Spread

If the mean or the median provides an estimate of a penny's expected mass, then the spread of individual measurements about the mean or median provides an estimate of the difference in mass among pennies or of the uncertainty in measuring mass with a balance. Although we often define the spread relative to a specific measure of central tendency, its magnitude is independent of the central value. Although shifting all measurements in the same direction by adding or subtracting a constant value changes the mean or median, it does not change the spread. There are three common measures of spread: the range, the standard deviation, and the variance.

Problem 13 at the end of the chapter asks you to show that this is true.

Range

The ***range***, *w*, is the difference between a data set's largest and smallest values.

$$w = X_{\text{largest}} - X_{\text{smallest}}$$

The range provides information about the total variability in the data set, but does not provide information about the distribution of individual values. The range for the data in Table 4.1.1 is

$$w = 3.198\ \text{g} - 3.056\ \text{g} = 0.142\ \text{g}$$

Standard Deviation

The ***standard deviation***, *s*, describes the spread of individual values about their mean, and is given as

$$s = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}} \qquad (4.1.1)$$

where X_i is one of the n individual values in the data set, and $\overline{X}$ is the data set's mean value. Frequently, we report the relative standard deviation, s_r, instead of the absolute standard deviation.

$$s_r = \frac{s}{\overline{X}}$$

The percent relative standard deviation, $\%s_r$, is $s_r \times 100$.

> The relative standard deviation is important because it allows for a more meaningful comparison between data sets when the individual measurements differ significantly in magnitude. Consider again the data in Table 4.1.1 . If we multiply each value by 10, the absolute standard deviation will increase by 10 as well; the relative standard deviation, however, is the same.

Example 4.1.3

Report the standard deviation, the relative standard deviation, and the percent relative standard deviation for the data in Table 4.1.1 ?

Solution

To calculate the standard deviation we first calculate the difference between each measurement and the data set's mean value (3.117), square the resulting differences, and add them together to find the numerator of Equation 4.1.1

$$\begin{aligned}(3.080 - 3.117)^2 &= (-0.037)^2 = 0.001369\\(3.094 - 3.117)^2 &= (-0.023)^2 = 0.000529\\(3.107 - 3.117)^2 &= (-0.010)^2 = 0.000100\\(3.056 - 3.117)^2 &= (-0.061)^2 = 0.003721\\(3.112 - 3.117)^2 &= (-0.005)^2 = 0.000025\\(3.174 - 3.117)^2 &= (+0.057)^2 = 0.003249\\(3.198 - 3.117)^2 &= (+0.081)^2 = \underline{0.006561}\\&\qquad\qquad\qquad\qquad\quad 0.015554\end{aligned}$$

For obvious reasons, the numerator of Equation 4.1.1 is called a sum of squares. Next, we divide this sum of squares by $n - 1$, where n is the number of measurements, and take the square root.

$$s = \sqrt{\frac{0.015554}{7 - 1}} = 0.051 \text{ g}$$

Finally, the relative standard deviation and percent relative standard deviation are

$$s_r = \frac{0.051 \text{ g}}{3.117 \text{ g}} = 0.016$$

$$\%s_r = (0.016) \times 100 = 1.6\%$$

It is much easier to determine the standard deviation using a scientific calculator with built in statistical functions.

Many scientific calculators include two keys for calculating the standard deviation. One key calculates the standard deviation for a data set of n samples drawn from a larger collection of possible samples, which corresponds to Equation 4.1.1The other key calculates the standard deviation for all possible samples. The latter is known as the population's standard deviation, which we will cover later in this chapter. Your calculator's manual will help you determine the appropriate key for each.

Variance

Another common measure of spread is the ***variance***, which is the square of the standard deviation. We usually report a data set's standard deviation, rather than its variance, because the mean value and the standard deviation share the same unit. As we will see shortly, the variance is a useful measure of spread because its values are additive.

Example 4.1.4

What is the variance for the data in Table 4.1.1 ?

Solution

The variance is the square of the absolute standard deviation. Using the standard deviation from Example 4.1.3 gives the variance as

$$s^2 = (0.051)^2 = 0.0026$$

? Exercise 4.1.1

The following data were collected as part of a quality control study for the analysis of sodium in serum; results are concentrations of Na^+ in mmol/L.

$$140 \quad 143 \quad 141 \quad 137 \quad 132 \quad 157 \quad 143 \quad 149 \quad 118 \quad 145$$

Report the mean, the median, the range, the standard deviation, and the variance for this data. This data is a portion of a larger data set from Andrew, D. F.; Herzberg, A. M. *Data: A Collection of Problems for the Student and Research Worker*, Springer-Verlag:New York, 1985, pp. 151–155.

Answer

Mean: To find the mean we add together the individual measurements and divide by the number of measurements. The sum of the 10 concentrations is 1405. Dividing the sum by 10, gives the mean as 140.5, or 1.40×10^2 mmol/L.

Median: To find the median we arrange the 10 measurements from the smallest concentration to the largest concentration; thus

$$118 \quad 132 \quad 137 \quad 140 \quad 141 \quad 143 \quad 143 \quad 145 \quad 149 \quad 157$$

The median for a data set with 10 members is the average of the fifth and sixth values; thus, the median is (141 + 143)/2, or 142 mmol/L.

Range: The range is the difference between the largest value and the smallest value; thus, the range is 157 – 118 = 39 mmol/L.

Standard Deviation: To calculate the standard deviation we first calculate the absolute difference between each measurement and the mean value (140.5), square the resulting differences, and add them together. The differences are

$$-0.5 \quad 2.5 \quad 0.5 \quad -3.5 \quad -8.5 \quad 16.5 \quad 2.5 \quad 8.5 \quad -22.5 \quad 4.5$$

and the squared differences are

$$0.25 \quad 6.25 \quad 0.25 \quad 12.25 \quad 72.25 \quad 272.25 \quad 6.25 \quad 72.25 \quad 506.25 \quad 20.25$$

The total sum of squares, which is the numerator of Equation 4.1.1, is 968.50. The standard deviation is

$$s = \sqrt{\frac{968.50}{10-1}} = 10.37 \approx 10.4$$

Variance: The variance is the square of the standard deviation, or 108.

4.2: Characterizing Experimental Errors

Characterizing a penny's mass using the data in Table 4.1.1 suggests two questions. First, does our measure of central tendency agree with the penny's expected mass? Second, why is there so much variability in the individual results? The first of these questions addresses the accuracy of our measurements and the second addresses the precision of our measurements. In this section we consider the types of experimental errors that affect accuracy and precision.

Errors That Affect Accuracy

Accuracy is how close a measure of central tendency is to its expected value, μ. We express accuracy either as an absolute error, *e*

$$e = \overline{X} - \mu \tag{4.2.1}$$

or as a percent relative error, %*e*

$$\%e = \frac{\overline{X} - \mu}{\mu} \times 100 \tag{4.2.2}$$

Although Equation 4.2.1 and Equation 4.2.2 use the mean as the measure of central tendency, we also can use the median.

The convention for representing a statistical parameter is to use a Roman letter for a value calculated from experimental data, and a Greek letter for its corresponding expected value. For example, the experimentally determined $\overline{X}$ mean is and its underlying expected value is μ. Likewise, the experimental standard deviation is *s* and the underlying expected value is σ.

We identify as determinate an error that affects the accuracy of an analysis. Each source of a ***determinate error*** has a specific magnitude and sign. Some sources of determinate error are positive and others are negative, and some are larger in magnitude and others are smaller in magnitude. The cumulative effect of these determinate errors is a net positive or negative error in accuracy.

It is possible, although unlikely, that the positive and negative determinate errors will offset each other, producing a result with no net error in accuracy.

We assign determinate errors into four categories—sampling errors, method errors, measurement errors, and personal errors—each of which we consider in this section.

Sampling Errors

A determinate sampling error occurs when our sampling strategy does not provide a us with a representative sample. For example, if we monitor the environmental quality of a lake by sampling from a single site near a point source of pollution, such as an outlet for industrial effluent, then our results will be misleading. To determine the mass of a U. S. penny, our strategy for selecting pennies must ensure that we do not include pennies from other countries.

An awareness of potential sampling errors especially is important when we work with heterogeneous materials. Strategies for obtaining representative samples are covered in Chapter 5.

Method Errors

In any analysis the relationship between the signal, S_{total}, and the absolute amount of analyte, n_A, or the analyte's concentration, C_A, is

$$S_{total} = k_A n_A + S_{mb} \tag{4.2.3}$$

$$S_{total} = k_A C_A + S_{mb} \tag{4.2.4}$$

Where k_A is the method's sensitivity for the analyte and S_{mb} is the signal from the method blank. A ***method error*** exists when our value for k_A or for S_{mb} is in error. For example, a method in which S_{total} is the mass of a precipitate assumes that k is defined by a pure precipitate of known stoichiometry. If this assumption is not true, then the resulting determination of n_A or C_A is inaccurate. We can minimize a determinate error in k_A by calibrating the method. A method error due to an interferent in the reagents is minimized by using a proper method blank.

Measurement Errors

The manufacturers of analytical instruments and equipment, such as glassware and balances, usually provide a statement of the item's

maximum ***measurement error***, or ***tolerance***. For example, a 10-mL volumetric pipet (Figure 4.2.1) has a tolerance of ±0.02 mL, which means the pipet delivers an actual volume within the range 9.98–10.02 mL at a temperature of 20 °C. Although we express this tolerance as a range, the error is determinate; that is, the pipet's expected volume, μ, is a fixed value within this stated range.

Figure 4.2.1 : Close-up of a 10-mL volumetric pipet showing that it has a tolerance of ±0.02 mL at 20 °C.

Volumetric glassware is categorized into classes based on its relative accuracy. Class A glassware is manufactured to comply with tolerances specified by an agency, such as the National Institute of Standards and Technology or the American Society for Testing and Materials. The tolerance level for Class A glassware is small enough that normally we can use it without calibration. The tolerance levels for Class B glassware usually are twice that for Class A glassware. Other types of volumetric glassware, such as beakers and graduated cylinders, are not used to measure volume accurately. Table 4.2.1 provides a summary of typical measurement errors for Class A volumetric glassware. Tolerances for digital pipets and for balances are provided in Table 4.2.2 and Table 4.2.3.

Table 4.2.1 : Measurement Errors for Type A Volumetric Glassware

Transfer Pipets		Volumetric Flasks		Burets	
Capacity (mL)	Tolerance (mL)	Capacity (mL)	Tolerance (mL)	Capacity (mL)	Tolerance (mL)
1	±0.006	5	±0.02	10	±0.02
2	±0.006	10	±0.02	25	±0.03
5	±0.01	25	±0.03	50	±0.05
10	±0.02	50	±0.05		
20	±0.03	100	±0.08		
25	±0.03	250	±0.12		
50	±0.05	500	±0.20		
100	±0.08	1000	±0.30		
		2000			

Table 4.2.2 : Measurement Errors for Digital Pipets

Pipet Range	Volume (mL or μL)	Percent Measurement Error
10–100 μL	10	±3.0%
	50	±1.0%
	100	±0.8%
100–1000 μL	100	±3.0%
	500	±1.0%
	1000	±0.6%
1–10 mL	1	±3.0%
	5	±0.8%
	10	±0.6%

Table 4.2.3 : Measurement Errors for Selected Balances

Balance	Capacity (g)	Measurement Error
Precisa 160M	160	±1 mg
A & D ER 120M	120	±0.1 mg
Metler H54	160	±0.01 mg

We can minimize a determinate measurement error by calibrating our equipment. Balances are calibrated using a reference weight whose mass we can trace back to the SI standard kilogram. Volumetric glassware and digital pipets are calibrated by determining the mass of water delivered or contained and using the density of water to calculate the actual volume. It is never safe to assume that a calibration does not change during an analysis or over time. One study, for example, found that repeatedly exposing volumetric glassware to higher temperatures during machine washing and oven drying, led to small, but significant changes in the glassware's calibration [Castanheira, I.; Batista, E.; Valente, A.; Dias, G.; Mora, M.; Pinto, L.; Costa, H. S. *Food Control* **2006**, *17*, 719–726]. Many instruments drift out of calibration over time and may require frequent recalibration during an analysis.

Personal Errors

Finally, analytical work is always subject to ***personal error***, examples of which include the ability to see a change in the color of an indicator that signals the endpoint of a titration, biases, such as consistently overestimating or underestimating the value on an instrument's readout scale, failing to calibrate instrumentation, and misinterpreting procedural directions. You can minimize personal errors by taking proper care.

Identifying Determinate Errors

Determinate errors often are difficult to detect. Without knowing the expected value for an analysis, the usual situation in any analysis that matters, we often have nothing to which we can compare our experimental result. Nevertheless, there are strategies we can use to detect determinate errors.

The magnitude of a constant ***determinate error*** is the same for all samples and is more significant when we analyze smaller samples. Analyzing samples of different sizes, therefore, allows us to detect a constant determinate error. For example, consider a quantitative analysis in which we separate the analyte from its matrix and determine its mass. Let's assume the sample is 50.0% w/w analyte. As we see in Table 4.2.4 , the expected amount of analyte in a 0.100 g sample is 0.050 g. If the analysis has a positive constant determinate error of 0.010 g, then analyzing the sample gives 0.060 g of analyte, or an apparent concentration of 60.0% w/w. As we increase the size of the sample the experimental results become closer to the expected result. An upward or downward trend in a graph of the analyte's experimental concentration versus the sample's mass (Figure 4.2.2) is evidence of a constant determinate error.

Table 4.2.4 : Effect of a Constant Determinate Error on the Analysis of a Sample That is 50.0% w/w Analyte

Mass of Sample (g)	Expected Mass of Analyte (g)	Constant Error (g)	Experimental Mass of Analyte (g)	Experimental Concentration of Analyte (% w/w)
0.100	0.050	0.010	0.060	60.0
0.200	0.100	0.010	0.110	55.0
0.400	0.200	0.010	0.210	52.5
0.800	0.400	0.010	0.410	51.2
1.600	0.800	0.010	0.810	50.6

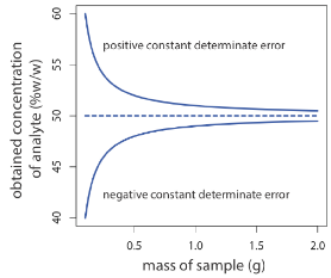


Figure 4.2.2 : Effect of a constant positive determinate error of +0.01 g and a constant negative determinate error of −0.01 g on the determination of an analyte in samples of varying size. The analyte's expected concentration of 50% w/w is shown by the dashed line.

A ***proportional determinate error***, in which the error's magnitude depends on the amount of sample, is more difficult to detect because the result of the analysis is independent of the amount of sample. Table 4.2.5 outlines an example that shows the effect of a positive proportional error of 1.0% on the analysis of a sample that is 50.0% w/w in analyte. Regardless of the sample's size, each analysis gives the same result of 50.5% w/w analyte.

Table 4.2.5 : Effect of a Proportional Determinate Error on the Analysis of a Sample That is 50.0% w/w Analyte

Mass of Sample (g)	Expected Mass of Analyte (g)	Proportional Error (%)	Experimental Mass of Analyte (g)	Experimental Concentration of Analyte (% w/w)
0.100	0.050	1.00	0.0505	50.5
0.200	0.100	1.00	0.101	50.5
0.400	0.200	1.00	0.202	50.5
0.800	0.400	1.00	0.404	50.5
1.600	0.800	1.00	0.808	50.5

One approach for detecting a proportional determinate error is to analyze

a standard that contains a known amount of analyte in a matrix similar to our samples. Standards are available from a variety of sources, such as the National Institute of Standards and Technology (where they are called Standard Reference Materials) or the American Society for Testing and Materials. Table 4.2.6 , for example, lists certified values for several analytes in a standard sample of *Gingko biloba* leaves. Another approach is to compare our analysis to an analysis carried out using an independent analytical method that is known to give accurate results. If the two methods give significantly different results, then a determinate error is the likely cause.

Table 4.2.6 : Certified Concentrations for SRM 3246: *Gingko bilbo* (Leaves)

Class of Analyte	Analyte	Mass Fraction (mg/g or ng/g)
Flavonoids/Ginkgolide B (mass fraction in mg/g)	Qurecetin	2.69 ± 0.31
	Kaempferol	3.02 ± 0.41
	Isorhamnetin	$0.517 \pm 0.0.99$
	Total Aglycones	6.22 ± 0.77
Selected Terpenes (mass fraction in mg/g)	Ginkgolide A	0.57 ± 0.28
	Ginkgolide B	0.470 ± 0.090
	Ginkgolide C	0.59 ± 0.22
	Ginkgolide J	0.18 ± 0.10
	Bilobalide	1.52 ± 0.40
	Total Terpene Lactones	3.3 ± 1.1
Selected Toxic Elements (mass fraction in ng/g)	Cadmium	20.8 ± 1.0
	Lead	995 ± 30
	Mercury	23.08 ± 0.17

The primary purpose of this Standard Reference Material is to validate analytical methods for determining flavonoids, terpene lactones, and toxic elements in *Ginkgo biloba* or other materials with a similar matrix. Values are from the official Certificate of Analysis available at www.nist.gov.

Constant and proportional determinate errors have distinctly different sources, which we can define in terms of the relationship between the signal and the moles or concentration of analyte (Equation 4.2.3 and Equation 4.2.4). An invalid method blank, S_{mb}, is a constant determinate error as it adds or subtracts the same value to the signal. A poorly calibrated method, which yields an invalid sensitivity for the analyte, k_A, results in a proportional determinate error.

Errors that Affect Precision

As we saw in Section 4.1, precision is a measure of the spread of individual measurements or results about a central value, which we express as a range, a standard deviation, or a variance. Here we draw a distinction between two types

of precision: repeatability and reproducibility. ***Repeatability*** is the precision when a single analyst completes an analysis in a single session using the same solutions, equipment, and instrumentation. ***Reproducibility***, on the other hand, is the precision under any other set of conditions, including between analysts or between laboratory sessions for a single analyst. Since reproducibility includes additional sources of variability, the reproducibility of an analysis cannot be better than its repeatability.

The ratio of the standard deviation associated with reproducibility to the standard deviation associated with repeatability is called the Horowitz ratio. For a wide variety of analytes in foods, for example, the median Horowtiz ratio is 2.0 with larger values for fatty acids and for trace elements; see Thompson, M.; Wood, R. "The 'Horowitz Ratio'–A Study of the Ratio Between Reproducibility and Repeatability in the Analysis of Foodstuffs," *Anal. Methods*, **2015**, *7*, 375–379.

Errors that affect precision are indeterminate and are characterized by random variations in their magnitude and their direction. Because they are random, positive and negative ***indeterminate errors*** tend to cancel, provided that we make a sufficient number of measurements. In such situations the mean and the median largely are unaffected by the precision of the analysis.

Sources of Indeterminate Error

We can assign indeterminate errors to several sources, including collecting samples, manipulating samples during the analysis, and making measurements. When we collect a sample, for instance, only a small portion of the available material is taken, which increases the chance that small-scale inhomogeneities in the sample will affect repeatability. Individual pennies, for example, may show variations in mass from several sources, including the manufacturing process and the loss of small amounts of metal or the addition of dirt during circulation. These variations are sources of indeterminate sampling errors.

During an analysis there are many opportunities to introduce indeterminate method errors. If our method for determining the mass of a penny includes directions for cleaning them of dirt, then we must be careful to treat each penny in the same way. Cleaning some pennies more vigorously than others might introduce an indeterminate method error.

Finally, all measuring devices are subject to indeterminate measurement errors due to limitations in our ability to read its scale. For example, a buret with scale divisions every 0.1 mL has an inherent indeterminate error of ±0.01–0.03 mL when we estimate the volume to the hundredth of a milliliter (Figure 4.2.3).

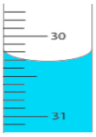


Figure 4.2.3 : Close-up of a buret showing the difficulty in estimating volume. With scale divisions every 0.1 mL it is difficult to read the actual volume to better than ±0.01–0.03 mL.

Evaluating Indeterminate Error

Indeterminate errors associated with our analytical equipment or instrumentation generally are easy to estimate if we measure the standard deviation for several replicate measurements, or if we monitor the signal's fluctuations over time in the absence of analyte (Figure 4.2.4) and calculate the standard deviation. Other sources of indeterminate error, such as treating samples inconsistently, are more difficult to estimate.

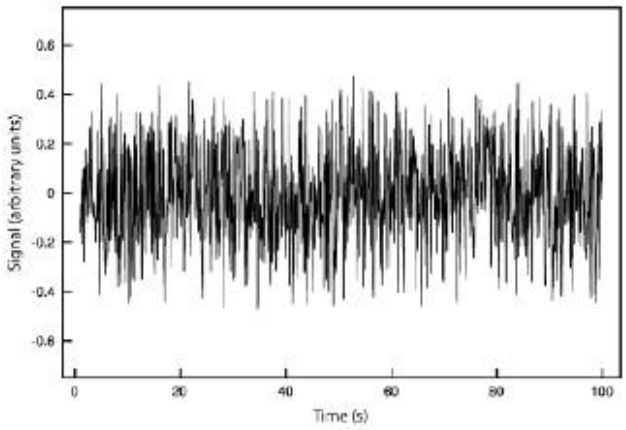


Figure 4.2.4 : Background noise in an instrument showing the random fluctuations in the signal.

To evaluate the effect of an indeterminate measurement error on our analysis of the mass of a circulating United States penny, we might make several determinations of the mass for a single penny (Table 4.2.7). The standard deviation for our original experiment (see Table 4.1.1) is 0.051 g, and it is 0.0024 g for the data in Table 4.2.7 . The significantly better precision when we determine the mass of a single penny suggests that the precision of our analysis is not limited by the balance. A more likely source of indeterminate error is a variability in the masses of individual pennies.

Table 4.2.7 : Replicate Determinations of the Mass of a Single Circulating U. S. Penny

Replicate	Mass (g)	Replicate	Mass (g)
1	3.025	6	3.023
2	3.024	7	3.022
3	3.028	8	3.021
4	3.027	9	3.026
5	3.028	10	3.024

In Section 4.5 we will discuss a statistical method—the *F*-test—that you can use to show that this difference is significant.

Error and Uncertainty

Analytical chemists make a distinction between error and uncertainty [Ellison, S.; Wegscheider, W.; Williams, A. *Anal. Chem.* **1997**, *69*, 607A–613A]. ***Error*** is the difference between a single measurement or result and its expected value. In other words, error is a measure of ***bias***. As discussed earlier, we divide errors into determinate and indeterminate sources. Although we can find and correct a source of determinate error, the indeterminate portion of the error remains.

Uncertainty expresses the range of possible values for a measurement or result. Note that this definition of uncertainty is not the same as our definition of precision. We calculate precision from our experimental data and use it to estimate the magnitude of indeterminate errors. Uncertainty accounts for all errors—both determinate and indeterminate—that reasonably might affect a measurement or a result. Although we always try to correct determinate errors before we begin an analysis, the correction itself is subject to uncertainty.

Here is an example to help illustrate the difference between precision and uncertainty. Suppose you purchase a 10-mL Class A pipet from a laboratory supply company and use it without any additional calibration. The pipet's tolerance of ±0.02 mL is its uncertainty because your best estimate of its expected volume is 10.00 mL ± 0.02 mL. This uncertainty primarily is determinate. If you use the pipet to dispense several replicate samples of a solution and determine the volume of each sample, the resulting standard deviation is the pipet's precision. Table 4.2.8 shows results for ten such trials, with a mean of 9.992 mL and a standard deviation of ±0.006 mL. This standard deviation is the precision with which we expect to deliver a solution using a Class A 10-mL pipet. In this case the pipet's published uncertainty of ±0.02 mL is worse than its experimentally determined precision of ±0.006 ml. Interestingly, the data in Table 4.2.8 allows us to calibrate this specific pipet's delivery volume as 9.992 mL. If we use this volume as a better estimate of the pipet's expected volume, then its uncertainty is ±0.006 mL. As expected, calibrating the pipet allows us to decrease its uncertainty [Kadis, R. *Talanta* **2004**, *64*, 167–173].

Table 4.2.8 : Experimental Results for Volume Dispensed by a 10–mL Class A Transfer Pipet

Replicate	Volume (ml)	Replicate	Volume (mL)
1	10.002	6	9.983
2	9.993	7	9.991
3	9.984	8	9.990
4	9.996	9	9.988
5	9.989	10	9.999

4.4: The Distribution of Measurements and Results

Earlier we reported results for a determination of the mass of a circulating United States penny, obtaining a mean of 3.117 g and a standard deviation of 0.051 g. Table 4.4.1 shows results for a second, independent determination of a penny's mass, as well as the data from the first experiment. Although the means and standard deviations for the two experiments are similar, they are not identical. The difference between the two experiments raises some interesting questions. Are the results for one experiment better than the results for the other experiment? Do the two experiments provide equivalent estimates for the mean and the standard deviation? What is our best estimate of a penny's expected mass? To answer these questions we need to understand how we might predict the properties of all pennies using the results from an analysis of a small sample of pennies. We begin by making a distinction between populations and samples.

Table 4.4.1 : Results for Two Determinations of the Mass of a Circulating U. S. Penny

First Experiment		Second Experiment	
Penny	Mass (g)	Penny	Mass (g)
1	3.080	1	3.052
2	3.094	2	3.141
3	3.107	3	3.083
4	3.056	4	3.083
5	3.112	5	3.048
6	3.174		
7	3.198		
$\overline{X}$	3.117		3.081
s	0.051		0.037

Populations and Samples

A ***population*** is the set of all objects in the system we are investigating. For the data in Table 4.4.1 , the population is all United States pennies in circulation. This population is so large that we cannot analyze every member of the population. Instead, we select and analyze a limited subset, or ***sample*** of the population. The data in Table 4.4.1 , for example, shows the results for two such samples drawn from the larger population of all circulating United States pennies.

Probability Distributions for Populations

Table 4.4.1 provides the means and the standard deviations for two samples of circulating United States pennies. What do these samples tell us about the population of pennies? What is the largest possible mass for a penny? What is

the smallest possible mass? Are all masses equally probable, or are some masses more common?

To answer these questions we need to know how the masses of individual pennies are distributed about the population's average mass. We represent the distribution of a population by plotting the probability or frequency of obtaining a specific result as a function of the possible results. Such plots are called ***probability distributions***.

There are many possible probability distributions; in fact, the probability distribution can take any shape depending on the nature of the population. Fortunately many chemical systems display one of several common probability distributions. Two of these distributions, the binomial distribution and the normal distribution, are discussed in this section.

The Binomial Distribution

The binomial distribution describes a population in which the result is the number of times a particular event occurs during a fixed number of trials. Mathematically, the binomial distribution is defined as

$$P(X,N) = \frac{N!}{X!(N-X)!} \times p^X \times (1-p)^{N-X}$$

where $P(X, N)$ is the probability that an event occurs X times during N trials, and p is the event's probability for a single trial. If you flip a coin five times, $P(2,5)$ is the probability the coin will turn up "heads" exactly twice.

> The term $N!$ reads as N-factorial and is the product $N \times (N-1) \times (N-2) \times \cdots \times 1$. For example, 4! is $4 \times 3 \times 2 \times 1 = 24$. Your calculator probably has a key for calculating factorials.

A binomial distribution has well-defined measures of central tendency and spread. The expected mean value is

$$\mu = Np$$

and the expected spread is given by the variance

$$\sigma^2 = Np(1-p)$$

or the standard deviation.

$$\sigma = \sqrt{Np(1-p)}$$

The binomial distribution describes a population whose members have only specific, discrete values. When you roll a die, for example, the possible values are 1, 2, 3, 4, 5, or 6. A roll of 3.45 is not possible. As shown in Worked Example 4.4.1 , one example of a chemical system that obeys the binomial distribution is the probability of finding a particular isotope in a molecule.

Example 4.4.1

Carbon has two stable, non-radioactive isotopes, ^{12}C and ^{13}C, with relative isotopic abundances of, respectively, 98.89% and 1.11%.

(a) What are the mean and the standard deviation for the number of ^{13}C atoms in a molecule of cholesterol ($C_{27}H_{44}O$)?

(b) What is the probability that a molecule of cholesterol has no atoms of ^{13}C?

Solution

The probability of finding an atom of ^{13}C in a molecule of cholesterol follows a binomial distribution, where X is the number of ^{13}C atoms, N is the number of carbon atoms in a molecule of cholesterol, and p is the probability that an atom of carbon in ^{13}C.

For (a), the mean number of ^{13}C atoms in a molecule of cholesterol is

$$\mu = Np = 27 \times 0.0111 = 0.300$$

with a standard deviation of

$$\sigma = \sqrt{Np(1-p)} = \sqrt{27 \times 0.0111 \times (1 - 0.0111)} = 0.544$$

For (b), the probability of finding a molecule of cholesterol without an atom of ^{13}C is

$$P(0, 27) = \frac{27!}{0!\,(27-0)!} \times (0.0111)^0 \times (1 - 0.0111)^{27-0} = 0.740$$

There is a 74.0% probability that a molecule of cholesterol will not have an atom of ^{13}C, a result consistent with the observation that the mean number of ^{13}C atoms per molecule of cholesterol, 0.300, is less than one.

A portion of the binomial distribution for atoms of ^{13}C in cholesterol is shown in Figure 4.4.1 . Note in particular that there is little probability of finding more than two atoms of ^{13}C in any molecule of cholesterol.

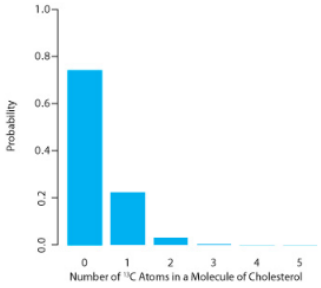


Figure 4.4.1 : Portion of the binomial distribution for the number of naturally occurring ^{13}C atoms in a molecule of cholesterol. Only 3.6% of cholesterol molecules contain more than one atom of ^{13}C, and only 0.33% contain more than two atoms of ^{13}C.

The Normal Distribution

A binomial distribution describes a population whose members have only certain discrete values. This is the case with the number of ^{13}C atoms in cholesterol. A molecule of cholesterol, for example, can have two ^{13}C atoms, but it can not have 2.5 atoms of ^{13}C. A population is continuous if its members may take on any value. The efficiency of extracting cholesterol from a sample, for example, can take on any value between 0% (no cholesterol is extracted) and 100% (all cholesterol is extracted).

The most common continuous distribution is the Gaussian, or ***normal distribution***, the equation for which is

$$f(X) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(X-\mu)^2}{2\sigma^2}}$$

where μ is the expected mean for a population with n members

$$\mu = \frac{\sum_{i=1}^{n} X_i}{n}$$

and σ^2 is the population's variance.

$$\sigma^2 = \frac{\sum_{i=1}^{n} (X_i - \mu)^2}{n} \tag{4.4.1}$$

Examples of three normal distributions, each with an expected mean of 0 and with variances of 25, 100, or 400, respectively, are shown in Figure 4.4.2 . Two

features of these normal distribution curves deserve attention. First, note that each normal distribution has a single maximum that corresponds to μ, and that the distribution is symmetrical about this value. Second, increasing the population's variance increases the distribution's spread and decreases its height; the area under the curve, however, is the same for all three distributions.

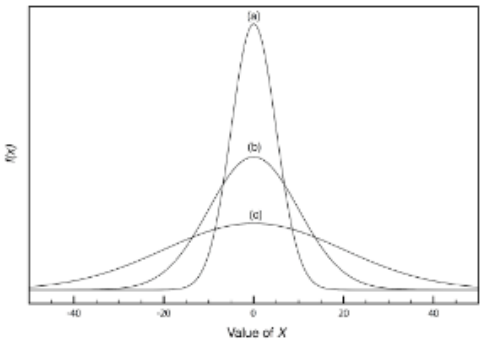


Figure 4.4.2 : Normal distribution curves for: (a) $\mu = 0$; $\sigma^2 = 25$ (b) $\mu = 0$; $\sigma^2 = 100$ (c) $\mu = 0$; $\sigma^2 = 400$.

The area under a normal distribution curve is an important and useful property as it is equal to the probability of finding a member of the population within a particular range of values. In Figure 4.4.2 , for example, 99.99% of the population shown in curve (a) have values of *X* between −20 and +20. For curve (c), 68.26% of the population's members have values of *X* between −20 and +20.

Because a normal distribution depends solely on μ and σ^2, the probability of finding a member of the population between any two limits is the same for all normally distributed populations. Figure 4.4.3 , for example, shows that 68.26% of the members of a normal distribution have a value within the range $\mu \pm 1\sigma$, and that 95.44% of population's members have values within the range $\mu \pm 2\sigma$. Only 0.27% members of a population have values that exceed the expected mean by more than ± 3σ. Additional ranges and probabilities are gathered together in the probability table included in Appendix 3. As shown in Example 4.4.2 , if we know the mean and the standard deviation for a normally distributed population, then we can determine the percentage of the population between any defined limits.

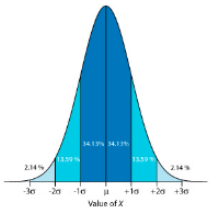


Figure 4.4.3 : Normal distribution curve showing the area under the curve for several different ranges of values of *X*.

Example 4.4.2

The amount of aspirin in the analgesic tablets from a particular manufacturer is known to follow a normal distribution with μ = 250 mg and σ = 5. In a random sample of tablets from the production line, what percentage are expected to contain between 243 and 262 mg of aspirin?

Solution

We do not determine directly the percentage of tablets between 243 mg and 262 mg of aspirin. Instead, we first find the percentage of tablets with less than 243 mg of aspirin and the percentage of tablets having more than 262 mg of aspirin. Subtracting these results from 100%, gives the percentage of tablets that contain between 243 mg and 262 mg of aspirin.

To find the percentage of tablets with less than 243 mg of aspirin or more than 262 mg of aspirin we calculate the deviation, *z*, of each limit from μ in terms of the population's standard deviation, σ

$$z = \frac{X - \mu}{\sigma}$$

where *X* is the limit in question. The deviation for the lower limit is

$$z_{lower} = \frac{243 - 250}{5} = -1.4$$

and the deviation for the upper limit is

$$z_{upper} = \frac{262 - 250}{5} = +2.4$$

Using the table in Appendix 3, we find that the percentage of tablets with less than 243 mg of aspirin is 8.08%, and that the percentage of tablets with more than 262 mg of aspirin is 0.82%. Therefore, the percentage of tablets containing between 243 and 262 mg of aspirin is

$$100.00\% - 8.08\% - 0.82\% = 91.10\%$$

Figure 4.4.4 shows the distribution of aspiring in the tablets, with the area in blue showing the percentage of tablets containing between 243 mg and 262 mg of aspirin.

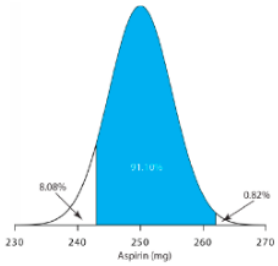


Figure 4.4.4 : Normal distribution for the population of aspirin tablets in Example 4.4.2 . The population's mean and standard deviation are 250 mg and 5 mg, respectively. The shaded area shows the percentage of tablets containing between 243 mg and 262 mg of aspirin.

? Exercise 4.4.1

What percentage of aspirin tablets will contain between 240 mg and 245 mg of aspirin if the population's mean is 250 mg and the population's standard deviation is 5 mg.

Answer

To find the percentage of tablets that contain less than 245 mg of aspirin we first calculate the deviation, z,

$$z = \frac{245 - 250}{5} = -1.00$$

and then look up the corresponding probability in Appendix 3, obtaining a value of 15.87%. To find the percentage of tablets that contain less than 240 mg of aspirin we find that

$$z = \frac{240 - 250}{5} = -2.00$$

which corresponds to 2.28%. The percentage of tablets containing between 240 and 245 mg of aspiring is 15.87% – 2.28% = 13.59%.

Confidence Intervals for Populations

If we select at random a single member from a population, what is its most likely value? This is an important question, and, in one form or another, it is at the heart of any analysis in which we wish to extrapolate from a sample to the sample's parent population. One of the most important features of a population's probability distribution is that it provides a way to answer this question.

Figure 4.4.3 shows that for a normal distribution, 68.26% of the population's members have values within the range $\mu \pm 1\sigma$. Stating this another way, there is a 68.26% probability that the result for a single sample drawn from a normally distributed population is in the interval $\mu \pm 1\sigma$. In general, if we select a single sample we expect its value, X_i is in the range

$$X_i = \mu \pm z\sigma \tag{4.4.2}$$

where the value of z is how confident we are in assigning this range. Values reported in this fashion are called ***confidence intervals***. Equation 4.4.2, for example, is the confidence interval for a single member of a population. Table 4.4.2 gives the confidence intervals for several values of z. For reasons discussed later in the chapter, a 95% confidence level is a common choice in analytical chemistry.

Table 4.4.2 : Confidence Intervals for a Normal Distribution

z	Confidence Interval
0.50	38.30
1.00	68.26
1.50	86.64
1.96	95.00
2.00	95.44
2.50	98.76
3.00	99.73
3.50	99.95

✔ Example 4.4.3

What is the 95% confidence interval for the amount of aspirin in a single analgesic tablet drawn from a population for which μ is 250 mg and for which σ is 5?

Solution

Using Table 4.4.2 , we find that z is 1.96 for a 95% confidence interval. Substituting this into Equation 4.4.2 gives the confidence interval for a single tablet as

$$X_i = \mu \pm 1.96\sigma = 250 \text{ mg} \pm (1.96 \times 5) = 250 \text{ mg} \pm 10\text{mg}$$

A confidence interval of 250 mg ± 10 mg means that 95% of the tablets in the population contain between 240 and 260 mg of aspirin.

Alternatively, we can rewrite Equation 4.4.2 so that it gives the confidence interval is for μ based on the population's standard deviation and the value of a single member drawn from the population.

$$\mu = X_i \pm z\sigma \tag{4.4.3}$$

✔ Example 4.4.4

The population standard deviation for the amount of aspirin in a batch of analgesic tablets is known to be 7 mg of aspirin. If you randomly select and analyze a single tablet and find that it contains 245 mg of aspirin, what is the 95% confidence interval for the population's mean?

Solution

The 95% confidence interval for the population mean is given as

$$\mu = X_i \pm z\sigma = 245 \text{ mg} \pm (1.96 \times 7) \text{ mg} = 245 \text{ mg} \pm 14 \text{ mg}$$

Therefore, based on this one sample, we estimate that there is 95% probability that the population's mean, μ, lies within the range of 231 mg to 259 mg of aspirin.

Note the qualification that the prediction for μ is based on one sample; a different sample likely will give a different 95% confidence interval. Our result here, therefore, is an estimate for μ based on this one sample.

It is unusual to predict the population's expected mean from the analysis of a single sample; instead, we collect n samples drawn from a population of known σ, and report the mean, $\overline{X}$. The standard deviation of the mean, $\sigma_{\overline{X}}$, which also is known as the ***standard error of the mean***, is

$$\sigma_{\overline{X}} = \frac{\sigma}{\sqrt{n}}$$

The confidence interval for the population's mean, therefore, is

$$\mu = \overline{X} \pm \frac{z\sigma}{\sqrt{n}}$$

✔ Example 4.4.5

What is the 95% confidence interval for the analgesic tablets in Example 4.4.4 , if an analysis of five tablets yields a mean of 245 mg of aspirin?

Solution

In this case the confidence interval is

$$\mu = 245 \text{ mg} \pm \frac{1.96 \times 7}{\sqrt{5}} \text{ mg} = 245 \text{ mg} \pm 6 \text{ mg}$$

We estimate a 95% probability that the population's mean is between 239 mg and 251 mg of aspirin. As expected, the confidence interval when using the mean of five samples is smaller than that for a single sample.

? Exercise 4.4.2

An analysis of seven aspirin tablets from a population known to have a standard deviation of 5, gives the following results in mg aspirin per tablet:

$$246 \quad 249 \quad 255 \quad 251 \quad 251 \quad 247 \quad 250$$

What is the 95% confidence interval for the population's expected mean?

Answer

The mean is 249.9 mg aspirin/tablet for this sample of seven tablets. For a 95% confidence interval the value of z is 1.96, which makes the confidence interval

$$249.9 \pm \frac{1.96 \times 5}{\sqrt{7}} = 249.9 \pm 3.7 \approx 250 \text{ mg} \pm 4 \text{ mg}$$

Probability Distributions for Samples

In Examples 4.4.2 –4.4.5 we assumed that the amount of aspirin in analgesic tablets is normally distributed. Without analyzing every member of the population, how can we justify this assumption? In a situation where we cannot study the whole population, or when we cannot predict the mathematical form of a population's probability distribution, we must deduce the distribution from a limited sampling of its members.

Sample Distributions and the Central Limit Theorem

Let's return to the problem of determining a penny's mass to explore further the relationship between a population's distribution and the distribution of a sample drawn from that population. The two sets of data in Table 4.4.1 are too small to provide a useful picture of a sample's distribution, so we will use the larger sample of 100 pennies shown in Table 4.4.3 . The mean and the standard deviation for this sample are 3.095 g and 0.0346 g, respectively.

Table 4.4.3 : Masses for a Sample of 100 Circulating U. S. Pennies

Penny	Weight (g)	Penny	Weight (g)	Penny	Weight (g)	Penny	Weight (g)
1	3.126	26	3.073	51	3.101	76	3.086
2	3.140	27	3.084	52	3.049	77	3.123
3	3.092	28	3.148	53	3.082	78	3.115
4	3.095	29	3.047	54	3.142	79	3.055
5	3.080	30	3.121	55	3.082	80	3.057
6	3.065	31	3.116	56	3.066	81	3.097
7	3.117	32	3.005	57	3.128	82	3.066
8	3.034	33	3.115	58	3.112	83	3.113
9	3.126	34	3.103	59	3.085	84	3.102
10	3.057	35	3.086	60	3.086	85	3.033
11	3.053	36	3.103	61	3.084	86	3.112
12	3.099	37	3.049	62	3.104	87	3.103

13	3.065	38	2.998	63	3.107	88	3.198
14	3.059	39	3.063	64	3.093	89	3.103
15	3.068	40	3.055	65	3.126	90	3.126
16	3.060	41	3.181	66	3.138	91	3.111
17	3.078	42	3.108	67	3.131	92	3.126
18	3.125	43	3.114	68	3.120	93	3.052
19	3.090	44	3.121	69	3.100	94	3.113
20	3.100	45	3.105	70	3.099	95	3.085
21	3.055	46	3.078	71	3.097	96	3.117
22	3.105	47	3.147	72	3.091	97	3.142
23	3.063	48	3.104	73	3.077	98	3.031
24	3.083	49	3.146	74	3.178	99	3.083
25	3.065	50	3.095	75	3.054	100	3.104

A ***histogram*** (Figure 4.4.5) is a useful way to examine the data in Table 4.4.3 . To create the histogram, we divide the sample into intervals, by mass, and determine the percentage of pennies within each interval (Table 4.4.4). Note that the sample's mean is the midpoint of the histogram.

Table 4.4.4 : Frequency Distribution for the Data in Table 4.4.3

Mass Interval	Frequency (as % of Sample)	Mass Interval	Frequency (as % of Sample)
2.991 – 3.009	2	3.105 – 3.123	19
3.010 – 3.028	0	3.124 – 3.142	12
3.029 – 3.047	4	3.143 – 3.161	3
3.048 – 3.066	19	3.162 – 3.180	1
3.067 – 3.085	14	3.181 – 3.199	2
3.086 – 3.104	24	3.200 – 3.218	0

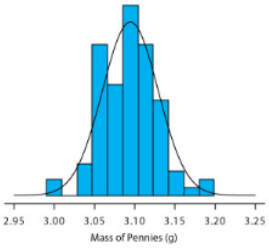


Figure 4.4.5 : The blue bars show a histogram for the data in Table 4.4.3 . The height of each bar corresponds to the percentage of pennies within one of the mass intervals in Table 4.4.4 . Superimposed on the histogram is a normal distribution curve based on the assumption that μ and σ^2 for the population are equivalent to $\overline{X}$ and σ^2 for the sample. The total area of the histogram's bars and the area under the normal distribution curve are equal.

Degrees of Freedom

Did you notice the differences between the equation for the variance of a population and the variance of a sample? If not, here are the two equations:

$$\sigma^2 = \frac{\sum_{i=1}^{n}(X_i - \mu)^2}{n}$$

$$s^2 = \frac{\sum_{i=1}^{n}(X_i - \overline{X})^2}{n-1}$$

Both equations measure the variance around the mean, using μ for a population and $\overline{X}$ for a sample. Although the equations use different measures for the mean, the intention is the same for both the sample and the population. A more interesting difference is between the denominators of the two equations. When we calculate the population's variance we divide the numerator by the population's size, n; for the sample's variance, however, we divide by $n - 1$, where n is the sample's size. Why do we divide by $n - 1$ when we calculate the sample's variance?

A variance is the average squared deviation of individual results relative to the mean. When we calculate an average we divide the sum by the number of independent measurements, or ***degrees of freedom***, in the calculation. For the population's variance, the degrees of freedom is equal to the population's size, n. When we measure every member of a population we have complete information about the population.

When we calculate the sample's variance, however, we replace μ with $\overline{X}$, which we also calculate using the same data. If there are n members in the sample, we can deduce the value of the n^{th} member from the remaining $n - 1$ members and the mean. For example, if \(n = 5\) and we know that the first four samples are 1, 2, 3 and 4, and that the mean is 3, then the fifth member of the sample must be

$$X_5 = (\overline{X} \times n) - X_1 - X_2 - X_3 - X_4 = (3 \times 5) - 1 - 2 - 3 - 4 = 5$$

Because we have just four independent measurements, we have lost one degree of freedom. Using $n - 1$ in place of n when we calculate the sample's variance ensures that s^2 is an unbiased estimator of σ^2.

Here is another way to think about degrees of freedom. We analyze samples to make predictions about the underlying population. When our sample consists of n measurements we cannot make more than n independent predictions about the population. Each time we estimate a parameter, such as the population's mean, we lose a degree of freedom. If there are n degrees of freedom for calculating the sample's mean, then $n - 1$ degrees of freedom remain when we calculate the sample's variance.

Confidence Intervals for Samples

Earlier we introduced the confidence interval as a way to report the most probable value for a population's mean, μ

$$\mu = \overline{X} \pm \frac{z\sigma}{\sqrt{n}} \tag{4.4.4}$$

where $\overline{X}$ is the mean for a sample of size n, and σ is the population's standard deviation. For most analyses we do not know the population's standard deviation. We can still calculate a confidence interval, however, if we make two modifications to Equation 4.4.4.

The first modification is straightforward—we replace the population's standard deviation, σ, with the sample's standard deviation, s. The second modification is not as obvious. The values of z in Table 4.4.2 are for a normal distribution, which is a function of $sigma^2$, not s^2. Although the sample's variance, s^2, is an unbiased estimate of the population's variance, σ^2, the value of s^2 will only rarely equal σ^2. To account for this uncertainty in estimating σ^2, we replace the variable z in Equation 4.4.4 with the variable t, where t is defined such that $t \geq z$ at all confidence levels.

$$\mu = \overline{X} \pm \frac{ts}{\sqrt{n}} \tag{4.4.5}$$

Values for t at the 95% confidence level are shown in Table 4.4.5 . Note that t becomes smaller as the number of degrees of freedom increases, and that it approaches z as n approaches infinity. The larger the sample, the more closely its confidence interval for a sample (Equation 4.4.5) approaches the confidence interval for the population (Equation 4.4.3). Appendix 4 provides additional values of t for other confidence levels.

Table 4.4.5 : Values of t for a 95% Confidence Interval

Degrees of Freedom	t	Degrees of Freedom	t	Degrees of Freedom	t	Degrees of Freedom	t
1	12.706	6	2.447	12	2.179	30	2.042
2	4.303	7	2.365	14	2.145	40	2.021
3	3.181	8	2.306	16	2.120	60	2.000
4	2.776	9	2.262	18	2.101	100	1.984
5	2.571	10	2.228	20	2.086	\(\infty	1.960

✔ Example 4.4.6

What are the 95% confidence intervals for the two samples of pennies in Table 4.4.1 ?

Solution

The mean and the standard deviation for first experiment are, respectively, 3.117 g and 0.051 g. Because the sample consists of seven measurements, there are six degrees of freedom. The value of t from Table 4.4.5 , is 2.447. Substituting into Equation 4.4.5 gives

$$\mu = 3.117\ \text{g} \pm \frac{2.447 \times 0.051\ \text{g}}{\sqrt{7}} = 3.117\ \text{g} \pm 0.047\ \text{g}$$

For the second experiment the mean and the standard deviation are 3.081 g and 0.073 g, respectively, with four degrees of freedom. The 95% confidence interval is

$$\mu = 3.081\ \text{g} \pm \frac{2.776 \times 0.037\ \text{g}}{\sqrt{5}} = 3.081\ \text{g} \pm 0.046\ \text{g}$$

Based on the first experiment, the 95% confidence interval for the population's mean is 3.070–3.164 g. For the second experiment, the 95% confidence interval is 3.035–3.127 g. Although the two confidence intervals are not identical—remember, each confidence interval provides a different estimate for μ—the mean for each experiment is contained within the other experiment's confidence interval. There also is an appreciable overlap of the two confidence intervals. Both of these observations are consistent with samples drawn from the same population.

Note that our comparison of these two confidence intervals at this point is somewhat vague and unsatisfying. We will return to this point in the next section, when we consider a statistical approach to comparing the results of experiments.

? Exercise 4.4.3

What is the 95% confidence interval for the sample of 100 pennies in Table 4.4.3 ? The mean and the standard deviation for this sample are 3.095 g and 0.0346 g, respectively. Compare your result to the confidence intervals for the samples of pennies in Table 4.4.1 .

Answer

With 100 pennies, we have 99 degrees of freedom for the mean. Although Table 4.4.3 does not include a value for $t(0.05, 99)$, we can approximate its value by using the values for $t(0.05, 60)$ and $t(0.05, 100)$ and by assuming a linear change in its value.

$$t(0.05, 99) = t(0.05, 60) - \frac{39}{40}\{t(0.05, 60) - t(0.05, 100\})$$

$$t(0.05, 99) = 2.000 - \frac{39}{40}\{2.000 - 1.984\} = 1.9844$$

The 95% confidence interval for the pennies is

$$3.095 \pm \frac{1.9844 \times 0.0346}{\sqrt{100}} = 3.095\ \text{g} \pm 0.007\ \text{g}$$

From Example 4.4.6 , the 95% confidence intervals for the two samples in Table 4.4.1 are 3.117 g ± 0.047 g and 3.081 g ± 0.046 g. As expected, the confidence interval for the sample of 100 pennies is much smaller than that for the two smaller samples of pennies. Note, as well, that the confidence interval for the larger sample fits within the confidence intervals for the two smaller samples.

A Cautionary Statement

There is a temptation when we analyze data simply to plug numbers into an equation, carry out the calculation, and report the result. This is never a good idea, and you should develop the habit of reviewing and evaluating your data. For example, if you analyze five samples and report an analyte's mean concentration as 0.67 ppm with a standard deviation of 0.64 ppm, then the 95% confidence interval is

$$\mu = 0.67 \text{ ppm} \pm \frac{2.776 \times 0.64 \text{ ppm}}{\sqrt{5}} = 0.67 \text{ ppm} \pm 0.79 \text{ ppm}$$

This confidence interval estimates that the analyte's true concentration is between −0.12 ppm and 1.46 ppm. Including a negative concentration within the confidence interval should lead you to reevaluate your data or your conclusions. A closer examination of your data may convince you that the standard deviation is larger than expected, making the confidence interval too broad, or you may conclude that the analyte's concentration is too small to report with confidence.

> We will return to the topic of detection limits near the end of this chapter.

Here is a second example of why you should closely examine your data: results obtained on samples drawn at random from a normally distributed population must be random. If the results for a sequence of samples show a regular pattern or trend, then the underlying population either is not normally distributed or there is a time-dependent determinate error. For example, if we randomly select 20 pennies and find that the mass of each penny is greater than that for the preceding penny, then we might suspect that our balance is drifting out of calibration.

5: Standardizing Analytical Methods

The American Chemical Society's Committee on Environmental Improvement defines standardization as the process of determining the relationship between the signal and the amount of analyte in a sample. In Chapter 3 we defined this relationship as

$$S_{total} = k_A n_A + S_{reag} \text{ or } S_{total} = k_A C_A + S_{reag}$$

where S_{total} is the signal, n_A is the moles of analyte, C_A is the analyte's concentration, k_A is the method's sensitivity for the analyte, and S_{reag} is the contribution to S_{total} from sources other than the sample. To standardize a method we must determine values for k_A and S_{reag}. Strategies for accomplishing this are the subject of this chapter.

5.1: Analytical Signals

To standardize an analytical method we use standards that contain known amounts of analyte. The accuracy of a standardization, therefore, depends on the quality of the reagents and the glassware we use to prepare these standards. For example, in an acid–base titration the stoichiometry of the acid–base reaction defines the relationship between the moles of analyte and the moles of titrant. In turn, the moles of titrant is the product of the titrant's concentration and the volume of titrant used to reach the equivalence point. The accuracy of a titrimetric analysis, therefore, is never better than the accuracy with which we know the titrant's concentration.

Primary and Secondary Standards

There are two categories of analytical standards: primary standards and secondary standards. A ***primary standard*** is a reagent that we can use to dispense an accurately known amount of analyte. For example, a 0.1250-g sample of $K_2Cr_2O_7$ contains 4.249×10^{-4} moles of $K_2Cr_2O_7$. If we place this sample in a 250-mL volumetric flask and dilute to volume, the concentration of $K_2Cr_2O_7$ in the resulting solution is 1.700×10^{-3} M. A primary standard must have a known stoichiometry, a known purity (or assay), and it must be stable during long-term storage. Because it is difficult to establishing accurately the degree of hydration, even after drying, a hydrated reagent usually is not a primary standard.

Reagents that do not meet these criteria are secondary standards. The concentration of a ***secondary standard*** is determined relative to a primary standard. Lists of acceptable primary standards are available (see, for instance, Smith, B. W.; Parsons, M. L. *J. Chem. Educ.* **1973**, *50*, 679–681; or Moody, J. R.; Green- burg, P.

> NaOH is one example of a secondary standard. Commercially available NaOH contains impurities of NaCl, Na_2CO_3, and Na_2SO_4, and readily absorbs H_2O from the atmosphere. To determine the concentration of NaOH in a solution, we titrate it against a primary standard weak acid, such as potassium hydrogen phthalate, $KHC_8H_4O_4$.

Other Reagents

Preparing a standard often requires additional reagents that are not primary standards or secondary standards, such as a suitable solvent or reagents needed to adjust the standard's matrix. These solvents and reagents are potential sources of additional analyte, which, if not accounted for, produce a determinate error in the standardization. If available, reagent grade chemicals that conform to standards set by the American Chemical Society are used [Committee on Analytical Reagents, *Reagent Chemicals*, 8th ed., American Chemical Society: Washington, D. C., 1993]. The label on the bottle of a reagent grade chemical (Figure 5.1.1) lists either the limits for specific impurities or provides an assay for the impurities. We can improve the quality of a reagent grade chemical by purifying it, or by conducting a more accurate assay. As discussed later in the chapter, we can correct for contributions to S_{total} from reagents used in an analysis by including an appropriate blank determination in the analytical procedure.

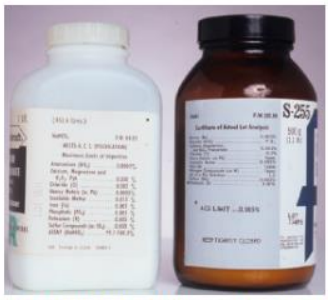


Figure 5.1.1 : Two examples of packaging labels for reagent grade chemicals. The label on the bottle on the right provides the manufacturer's assay for the reagent, NaBr. Note that potassium is flagged with an asterisk (*) because its assay exceeds the limit established by the American Chemical Society (ACS). The label for the bottle on the left does not provide an assay for impurities; however it indicates that the reagent meets ACS specifications by providing the maximum limits for impurities. An assay for the reagent, $NaHCO_3$, is provided.

Preparing a Standard Solution

It often is necessary to prepare a series of standards, each with a different concentration of analyte. We can prepare these standards in two ways. If the range of concentrations is limited to one or two orders of magnitude, then each solution is best prepared by transferring a known mass or volume of the pure standard to a volumetric flask and diluting to volume.

When working with a larger range of concentrations, particularly a range that extends over more than three orders of magnitude, standards are best prepared by a serial dilution from a single stock solution. In a ***serial dilution*** we prepare the most concentrated standard and then dilute a portion of that solution to prepare the next most concentrated standard. Next, we dilute a portion of the second standard to prepare a third standard, continuing this process until we have prepared all of our standards. Serial dilutions must be prepared with extra care because an error in preparing one standard is passed on to all succeeding standards.

5.2: Calibrating the Signal

The accuracy with which we determine k_A and S_{reag} depends on how accurately we can measure the signal, S_{total}. We measure signals using equipment, such as glassware and balances, and instrumentation, such as spectrophotometers and pH meters. To minimize determinate errors that might affect the signal, we first calibrate our equipment and instrumentation by measuring S_{total} for a standard with a known response of S_{std}, adjusting S_{total} until

$$S_{total} = S_{std}$$

Here are two examples of how we calibrate signals; other examples are provided in later chapters that focus on specific analytical methods.

When the signal is a measurement of mass, we determine S_{total} using an analytical balance. To calibrate the balance's signal we use a reference weight that meets standards established by a governing agency, such as the National Institute for Standards and Technology or the American Society for Testing and Materials. An electronic balance often includes an internal calibration weight for routine calibrations, as well as programs for calibrating with external weights. In either case, the balance automatically adjusts S_{total} to match S_{std}.

We also must calibrate our instruments. For example, we can evaluate a spectrophotometer's accuracy by measuring the absorbance of a carefully prepared solution of 60.06 mg/L $K_2Cr_2O_7$ in 0.0050 M H_2SO_4, using 0.0050 M H_2SO_4 as a reagent blank [Ebel, S. *Fresenius J. Anal. Chem.* **1992**, *342*, 769]. An absorbance of \(0.640 \pm 0.010\) absorbance units at a wavelength of 350.0 nm indicates that the spectrometer's signal is calibrated properly.

> Be sure to read and follow carefully the calibration instructions provided with any instrument you use.

5.3: Determining the Sensitivity

To standardize an analytical method we also must determine the analyte's sensitivity, k_A, in Equation 5.3.1 or Equation 5.3.2 .

$$S_{total} = k_A n_A + S_{reag} \tag{5.3.1}$$

$$S_{total} = k_A C_A + S_{reag} \tag{5.3.2}$$

In principle, it is possible to derive the value of k_A for any analytical method if we understand fully all the chemical reactions and physical processes responsible for the signal. Unfortunately, such calculations are not feasible if we lack a sufficiently developed theoretical model of the physical processes or if the chemical reaction's evince non-ideal behavior. In such situations we must determine the value of k_A by analyzing one or more standard solutions, each of which contains a known amount of analyte. In this section we consider several approaches for determining the value of k_A. For simplicity we assume that S_{reag} is accounted for by a proper reagent blank, allowing us to replace S_{total} in Equation 5.3.1 and Equation 5.3.2 with the analyte's signal, S_A.

$$S_A = k_A n_A \tag{5.3.3}$$

$$S_A = k_A C_A \tag{5.3.4}$$

> Equation 5.3.3 and Equation 5.3.4 essentially are identical, differing only in whether we choose to express the amount of analyte in moles or as a concentration. For the remainder of this chapter we will limit our treatment to Equation 5.3.4. You can extend this treatment to Equation 5.3.3 by replacing C_A with n_A.

Single-Point versus Multiple-Point Standardizations

The simplest way to determine the value of k_A in Equation 5.3.4 is to use a single-point standardization in which we measure the signal for a standard, S_{std}, that contains a known concentration of analyte, C_{std}. Substituting these values into Equation 5.3.4

$$k_A = \frac{S_{std}}{C_{std}} \tag{5.3.5}$$

gives us the value for k_A. Having determined k_A, we can calculate the concentration of analyte in a sample by measuring its signal, S_{samp}, and calculating C_A using Equation 5.3.6.

$$C_A = \frac{S_{samp}}{k_A} \tag{5.3.6}$$

A single-point standardization is the least desirable method for standardizing a method. There are two reasons for this. First, any error in our determination of k_A carries over into our calculation of C_A. Second, our experimental value for k_A is based on a single concentration of analyte. To extend this value of k_A to other concentrations of analyte requires that we assume a linear relationship between the signal and the analyte's concentration, an assumption that often is not true [Cardone, M. J.; Palmero, P. J.; Sybrandt, L. B. *Anal. Chem.* **1980**, *52*, 1187–1191]. Figure 5.3.1 shows how assuming a constant value of k_A leads to a determinate error in C_A if k_A becomes smaller at higher concentrations of analyte. Despite these limitations, single-point standardizations find routine use when the expected range for the analyte's concentrations is small. Under these conditions it often is safe to assume that k_A is constant (although you should verify this assumption experimentally). This is the case, for example, in clinical labs where many automated analyzers use only a single standard.

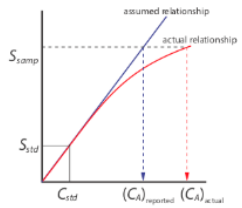


Figure 5.3.1 : Example showing how a single-point standardization leads to a determinate error in an analyte's reported concentration if we incorrectly assume that k_A is constant. The assumed relationship between S_{samp} and C_A is based on a single standard and is a straight-line; the actual relationship between S_{samp} and C_A becomes curved for larger concentrations of analyte.

The better way to standardize a method is to prepare a series of standards, each of which contains a different concentration of analyte. Standards are chosen such that they bracket the expected range for the analyte's concentration. A ***multiple-point standardization*** should include at least three standards, although more are preferable. A plot of S_{std} versus C_{std} is called a ***calibration curve***. The exact standardization, or calibration relationship, is determined by an appropriate curve-fitting algorithm.

> Linear regression, which also is known as the method of least squares, is one such algorithm. Its use is covered in Section 5.4.

There are two advantages to a multiple-point standardization. First, although a determinate error in one standard introduces a determinate error, its effect is minimized by the remaining standards. Second, because we measure the signal for several concentrations of analyte, we no longer must assume k_A is independent of the analyte's concentration. Instead, we can construct a calibration curve similar to the "actual relationship" in Figure 5.3.1 .

External Standards

The most common method of standardization uses one or more ***external standards***, each of which contains a known concentration of analyte. We call these standards "external" because they are prepared and analyzed separate from the samples.

> Appending the adjective "external" to the noun "standard" might strike you as odd at this point, as it seems reasonable to assume that standards and samples are analyzed separately. As we will soon learn, however, we can add standards to our samples and analyze both simultaneously.

Single External Standard

With a single external standard we determine k_A using EEquation 5.3.5 and then calculate the concentration of analyte, C_A, using Equation 5.3.6.

✔ Example 5.3.1

A spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{std} of 0.474 for a single standard for which the concentration of lead is 1.75 ppb. What is the concentration of Pb^{2+} in a sample of blood for which S_{samp} is 0.361?

Solution

Equation 5.3.5 allows us to calculate the value of k_A using the data for the single external standard.

$$k_A = \frac{S_{std}}{C_{std}} = \frac{0.474}{1.75 \text{ ppb}} = 0.2709 \text{ ppb}^{-1}$$

Having determined the value of k_A, we calculate the concentration of Pb^{2+} in the sample of blood is calculated using Equation 5.3.6.

$$C_A = \frac{S_{samp}}{k_A} = \frac{0.361}{0.2709 \text{ ppb}^{-1}} = 1.33 \text{ ppb}$$

Multiple External Standards

Figure 5.3.2 shows a typical multiple-point external standardization. The volumetric flask on the left contains a reagent blank and the remaining volumetric flasks contain increasing concentrations of Cu^{2+}. Shown below the volumetric flasks is the resulting calibration curve. Because this is the most common method of standardization, the resulting relationship is called a ***normal calibration curve***.

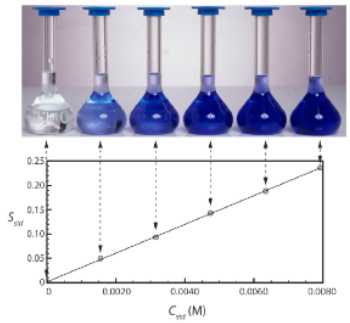


Figure 5.3.2 : The photo at the top of the figure shows a reagent blank (far left) and a set of five external standards for Cu^{2+} with concentrations that increase from left-to-right. Shown below the external standards is the resulting normal calibration curve. The absorbance of each standard, S_{std}, is shown by the filled circles.

When a calibration curve is a straight-line, as it is in Figure 5.3.2 , the slope of the line gives the value of k_A. This is the most desirable situation because the method's sensitivity remains constant throughout the analyte's concentration range. When the calibration curve is not a straight-line, the method's sensitivity is a function of the analyte's concentration. In Figure 5.3.1 , for example, the value of k_A is greatest when the analyte's concentration is small and it decreases continuously for higher concentrations of analyte. The value of k_A at any point along the calibration curve in Figure 5.3.1 is the slope at that point. In either case, a calibration curve allows to relate S_{samp} to the analyte's concentration.

Example 5.3.2

A second spectrophotometric method for the quantitative analysis of Pb^{2+} in blood has a normal calibration curve for which

$$S_{std} = (0.296\ \text{ppb}^{-1} \times C_{std}) + 0.003$$

What is the concentration of Pb^{2+} in a sample of blood if S_{samp} is 0.397?

Solution

To determine the concentration of Pb^{2+} in the sample of blood, we replace S_{std} in the calibration equation with S_{samp} and solve for C_A.

$$C_A = \frac{S_{samp} - 0.003}{0.296\ \text{ppb}^{-1}} = \frac{0.397 - 0.003}{0.296\ \text{ppb}^{-1}} = 1.33\ \text{ppb}$$

It is worth noting that the calibration equation in this problem includes an extra term that does not appear in Equation 5.3.6. Ideally we expect our calibration curve to have a signal of zero when C_A is zero. This is the purpose of using a reagent blank to correct the measured signal. The extra term of +0.003 in our calibration equation results from the uncertainty in measuring the signal for the reagent blank and the standards.

Exercise 5.3.1

Figure 5.3.2 shows a normal calibration curve for the quantitative analysis of Cu^{2+}. The equation for the calibration curve is

$$S_{std} = 29.59\ \text{M}^{-1} \times C_{std} + 0.015$$

What is the concentration of Cu^{2+} in a sample whose absorbance, S_{samp}, is 0.114? Compare your answer to a one-point standardization where a standard of 3.16×10^{-3} M Cu^{2+} gives a signal of 0.0931.

Answer

Substituting the sample's absorbance into the calibration equation and solving for C_A give

$$S_{samp} = 0.114 = 29.59\ \text{M}^{-1} \times C_A + 0.015$$

$$C_A = 3.35 \times 10^{-3}\ \text{M}$$

For the one-point standardization, we first solve for k_A

$$k_A = \frac{S_{std}}{C_{std}} = \frac{0.0931}{3.16 \times 10^{-3}\ \text{M}} = 29.46\ \text{M}^{-1}$$

and then use this value of k_A to solve for C_A.

$$C_A = \frac{S_{samp}}{k_A} = \frac{0.114}{29.46\ \text{M}^{-1}} = 3.87 \times 10^{-3}\ \text{M}$$

When using multiple standards, the indeterminate errors that affect the signal for one standard are partially compensated for by the indeterminate errors that affect the other standards. The standard selected for the one-point standardization has a signal that is smaller than that predicted by the regression equation, which underestimates k_A and overestimates C_A.

An external standardization allows us to analyze a series of samples using a single calibration curve. This is an important advantage when we have many samples to analyze. Not surprisingly, many of the most common quantitative analytical methods use an external standardization.

There is a serious limitation, however, to an external standardization. When we determine the value of k_A using Equation 5.3.5, the analyte is present in the external standard's matrix, which usually is a much simpler matrix than that of our samples. When we use an external standardization we assume the matrix

does not affect the value of k_A. If this is not true, then we introduce a proportional determinate error into our analysis. This is not the case in Figure 5.3.3 , for instance, where we show calibration curves for an analyte in the sample's matrix and in the standard's matrix. In this case, using the calibration curve for the external standards leads to a negative determinate error in analyte's reported concentration. If we expect that matrix effects are important, then we try to match the standard's matrix to that of the sample, a process known as ***matrix matching***. If we are unsure of the sample's matrix, then we must show that matrix effects are negligible or use an alternative method of standardization. Both approaches are discussed in the following section.

> The matrix for the external standards in Figure 5.3.2 , for example, is dilute ammonia. Because the $Cu(NH_3)_4^{2+}$ complex absorbs more strongly than Cu^{2+}, adding ammonia increases the signal's magnitude. If we fail to add the same amount of ammonia to our samples, then we will introduce a proportional determinate error into our analysis.

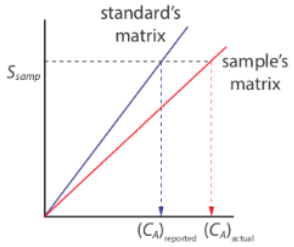


Figure 5.3.3 : Calibration curves for an analyte in the standard's matrix and in the sample's matrix. If the matrix affects the value of k_A, as is the case here, then we introduce a proportional determinate error into our analysis if we use a normal calibration curve.

Standard Additions

We can avoid the complication of matching the matrix of the standards to the matrix of the sample if we carry out the standardization in the sample. This is known as the ***method of standard additions***.

Single Standard Addition

The simplest version of a standard addition is shown in Figure 5.3.4 . First we add a portion of the sample, V_o, to a volumetric flask, dilute it to volume, V_f, and measure its signal, S_{samp}. Next, we add a second identical portion of sample to an equivalent volumetric flask along with a spike, V_{std}, of an external standard whose concentration is C_{std}. After we dilute the spiked sample to the same final volume, we measure its signal, S_{spike}.

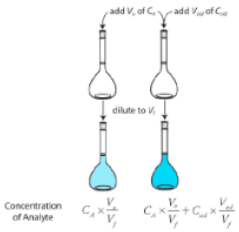


Figure 5.3.4 : Illustration showing the method of standard additions. The volumetric flask on the left contains a portion of the sample, V_o, and the volumetric flask on the right contains an identical portion of the sample and a spike, V_{std}, of a standard solution of the analyte. Both flasks are diluted to the same final volume, V_f. The concentration of analyte in each flask is shown at the bottom of the figure where C_A is the analyte's concentration in the original sample and C_{std} is the concentration of analyte in the external standard.

The following two equations relate S_{samp} and S_{spike} to the concentration of analyte, C_A, in the original sample.

$$S_{samp} = k_A C_A \frac{V_o}{V_f} \tag{5.3.7}$$

$$S_{spike} = k_A \left(C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f} \right) \tag{5.3.8}$$

As long as V_{std} is small relative to V_o, the effect of the standard's matrix on the sample's matrix is insignificant. Under these conditions the value of k_A is the same in Equation 5.3.7 and Equation 5.3.8. Solving both equations for k_A and equating gives

$$\frac{S_{samp}}{C_A \frac{V_o}{V_f}} = \frac{S_{spike}}{C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f}} \tag{5.3.9}$$

which we can solve for the concentration of analyte, C_A, in the original sample.

Example 5.3.3

A third spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{samp} of 0.193 when a 1.00 mL sample of blood is diluted to 5.00 mL. A second 1.00 mL sample of blood is spiked with 1.00 mL of a 1560-ppb Pb^{2+} external standard and diluted to 5.00 mL, yielding an S_{spike} of 0.419. What is the concentration of Pb^{2+} in the original sample of blood?

Solution

We begin by making appropriate substitutions into Equation 5.3.9 and solving for C_A. Note that all volumes must be in the same units; thus, we first convert V_{std} from 1.00 mL to 1.00×10^{-3} mL.

$$\frac{0.193}{C_A \frac{1.00\text{ mL}}{5.00\text{ mL}}} = \frac{0.419}{C_A \frac{1.00\text{ mL}}{5.00\text{ mL}} + 1560\text{ ppb}\frac{1.00\times10^{-3}\text{ mL}}{5.00\text{ mL}}}$$

$$\frac{0.193}{0.200C_A} = \frac{0.419}{0.200C_A + 0.3120\text{ ppb}}$$

$$0.0386C_A + 0.0602\text{ ppb} = 0.0838C_A$$

$$0.0452C_A = 0.0602\text{ ppb}$$

$$C_A = 1.33\text{ ppb}$$

The concentration of Pb^{2+} in the original sample of blood is 1.33 ppb.

It also is possible to add the standard addition directly to the sample, measuring the signal both before and after the spike (Figure 5.3.5). In this case the final volume after the standard addition is $V_o + V_{std}$ and Equation 5.3.7, Equation 5.3.8, and Equation 5.3.9 become

$$S_{samp} = k_A C_A$$

$$S_{spike} = k_A \left(C_A \frac{V_o}{V_o + V_{std}} + C_{std} \frac{V_{std}}{V_o + V_{std}} \right) \tag{5.3.10}$$

$$\frac{S_{samp}}{C_A} = \frac{S_{spike}}{C_A \frac{V_o}{V_o+V_{std}} + C_{std} \frac{V_{std}}{V_o+V_{std}}} \tag{5.3.11}$$

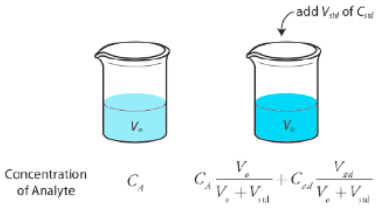


Figure 5.3.5 : Illustration showing an alternative form of the method of standard additions. In this case we add the spike of external standard directly to the sample without any further adjust in the volume.

Example 5.3.4

A fourth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood yields an S_{samp} of 0.712 for a 5.00 mL sample of blood. After spiking the blood sample with 5.00 mL of a 1560-ppb Pb^{2+} external standard, an S_{spike} of 1.546 is measured. What is the concentration of Pb^{2+} in the original sample of blood?

Solution

$$\frac{0.712}{C_A} = \frac{1.546}{C_A \frac{5.00\text{ mL}}{5.005\text{ mL}} + 1560\text{ ppb}\frac{5.00\times10^{-3}\text{ mL}}{5.005\text{ mL}}}$$

$$\frac{0.712}{C_A} = \frac{1.546}{0.9990C_A + 1.558\text{ ppb}}$$

$$0.7113C_A + 1.109\text{ ppb} = 1.546C_A$$

$$C_A = 1.33\text{ ppb}$$

The concentration of Pb^{2+} in the original sample of blood is 1.33 ppb.

Multiple Standard Additions

We can adapt a single-point standard addition into a multiple-point standard addition by preparing a series of samples that contain increasing amounts of the external standard. Figure 5.3.6 shows two ways to plot a standard addition calibration curve based on Equation 5.3.8. In Figure 5.3.6 a we plot S_{spike} against the volume of the spikes, V_{std}. If k_A is constant, then the calibration curve is a straight-line. It is easy to show that the x-intercept is equivalent to $-C_A V_o / C_{std}$.

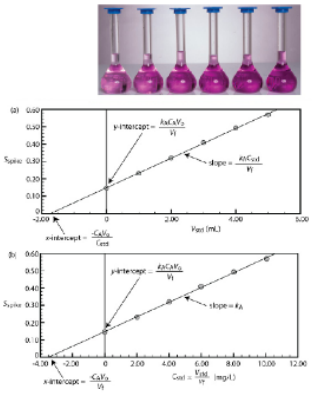


Figure 5.3.6 : Shown at the top of the figure is a set of six standard additions for the determination of Mn^{2+}. The flask on the left is a 25.00 mL sample diluted to 50.00 mL with water. The remaining flasks contain 25.00 mL of sample and, from left-to-right, 1.00, 2.00, 3.00, 4.00, and 5.00 mL spikes of an external standard that is 100.6 mg/L Mn^{2+}. Shown below are two ways to plot the standard additions calibration curve. The absorbance for each standard addition, S_{spike}, is shown by the filled circles.

✔ Example 5.3.5

Beginning with Equation 5.3.8 show that the equations in Figure 5.3.6 a for the slope, the *y*-intercept, and the *x*-intercept are correct.

Solution

We begin by rewriting Equation 5.3.8 as

$$S_{spike} = \frac{k_A C_A V_o}{V_f} + \frac{k_A C_{std}}{V_f} \times V_{std}$$

which is in the form of the equation for a straight-line

$$y = y\text{-intercept} + \text{slope} \times x\text{-intercept}$$

where *y* is S_{spike} and *x* is V_{std}. The slope of the line, therefore, is $k_A C_{std} / V_f$ and the *y*-intercept is $k_A C_A V_o / V_f$. The *x*-intercept is the value of *x* when *y* is zero, or

$$0 = \frac{k_A C_A V_o}{V_f} + \frac{k_A C_{std}}{V_f} \times x\text{-intercept}$$

$$x\text{-intercept} = -\frac{k_A C_A V_o / V_f}{K_A C_{std} / V_f} = -\frac{C_A V_o}{C_{std}}$$

? Exercise 5.3.2

Beginning with Equation 5.3.8 show that the Equations in Figure 5.3.6 b for the slope, the *y*-intercept, and the *x*-intercept are correct.

Answer

We begin with Equation 5.3.8

$$S_{spike} = k_A \left(C_A \frac{V_o}{V_f} + C_{std} \frac{V_{std}}{V_f} \right)$$

rewriting it as

$$S_{spike} = \frac{k_A C_A V_o}{V_f} + k_A \left(C_{std} \frac{V_{std}}{V_f} \right)$$

which is in the form of the linear equation

$$y = y\text{-intercept} + \text{slope} \times x\text{-intercept}$$

where *y* is S_{spike} and *x* is $C_{std} \times V_{std} / V_f$. The slope of the line, therefore, is k_A, and the *y*-intercept is $k_A C_A V_o / V_f$. The *x*-intercept is the value of *x* when *y* is zero, or

$$x\text{-intercept} = -\frac{k_A C_A V_o / V_F}{k_A} = -\frac{C_A V_o}{V_f}$$

Because we know the volume of the original sample, V_o, and the concentration of the external standard, C_{std}, we can calculate the analyte's concentrations from the *x*-intercept of a multiple-point standard additions.

✔ Example 5.3.6

A fifth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood uses a multiple-point standard addition based on Equation 5.3.8. The original blood sample has a volume of 1.00 mL and the standard used for spiking the sample has a concentration of 1560 ppb Pb^{2+}. All samples were diluted to 5.00 mL before measuring the signal. A calibration curve of S_{spike} versus V_{std} has the following equation

$$S_{spike} = 0.266 + 312\ \text{mL}^{-1} \times V_{std}$$

What is the concentration of Pb^{2+} in the original sample of blood?

Solution

To find the *x*-intercept we set S_{spike} equal to zero.

$$S_{spike} = 0.266 + 312\ \text{mL}^{-1} \times V_{std}$$

Solving for V_{std}, we obtain a value of -8.526×10^{-4} mL for the *x*-intercept. Substituting the *x*-intercept's value into the equation from Figure 5.3.6 a

$$-8.526 \times 10^{-4}\ \text{mL} = -\frac{C_A V_o}{C_{std}} = -\frac{C_A \times 1.00\ \text{mL}}{1560\ \text{ppb}}$$

and solving for C_A gives the concentration of Pb^{2+} in the blood sample as 1.33 ppb.

? Exercise 5.3.3

Figure 5.3.6 shows a standard additions calibration curve for the quantitative analysis of Mn^{2+}. Each solution contains 25.00 mL of the original sample and either 0, 1.00, 2.00, 3.00, 4.00, or 5.00 mL of a 100.6 mg/L external standard of Mn^{2+}. All standard addition samples were diluted to 50.00 mL with water before reading the absorbance. The equation for the calibration curve in Figure 5.3.6 a is

$$S_{std} = 0.0854 \times V_{std} + 0.1478$$

What is the concentration of Mn^{2+} in this sample? Compare your answer to the data in Figure 5.3.6 b, for which the calibration curve is

$$S_{std} = 0.425 \times C_{std}(V_{std}/V_f) + 0.1478$$

Answer

Using the calibration equation from Figure 5.3.6 a, we find that the *x*-intercept is

$$x\text{-intercept} = -\frac{0.1478}{0.0854\ \text{mL}^{-1}} = -1.731\ \text{mL}$$

If we plug this result into the equation for the *x*-intercept and solve for C_A, we find that the concentration of Mn^{2+} is

$$C_A = -\frac{x\text{-intercept} \times C_{std}}{V_o} = -\frac{-1.731\ \text{mL} \times 100.6\ \text{mg/L}}{25.00\ \text{mL}} = 6.96\ \text{mg/L}$$

For Figure 5.3.6 b, the *x*-intercept is

$$x\text{-intercept} = -\frac{0.1478}{0.0425\ \text{mL/mg}} = -3.478\ \text{mg/mL}$$

and the concentration of Mn^{2+} is

$$C_A = -\frac{x\text{-intercept} \times V_f}{V_o} = -\frac{-3.478\ \text{mg/mL} \times 50.00\ \text{mL}}{25.00\ \text{mL}} = 6.96\ \text{mg/L}$$

Since we construct a standard additions calibration curve in the sample, we can not use the calibration equation for other samples. Each sample, therefore, requires its own standard additions calibration curve. This is a serious drawback if you have many samples. For example, suppose you need to analyze 10 samples using a five-point calibration curve. For a normal calibration curve you need to analyze only 15 solutions (five standards and ten samples). If you use the method of standard additions, however, you must analyze 50 solutions (each of the ten samples is analyzed five times, once before spiking and after each of four spikes).

Using a Standard Addition to Identify Matrix Effects

We can use the method of standard additions to validate an external standardization when matrix matching is not feasible. First, we prepare a normal calibration curve of S_{std} versus C_{std} and determine the value of k_A from its slope. Next, we prepare a standard additions calibration curve using Equation 5.3.8, plotting the data as shown in Figure 5.3.6 b. The slope of this standard additions calibration curve provides an independent determination of k_A. If there is no significant difference between the two values of k_A, then we can ignore the difference between the sample's matrix and that of the external standards. When the values of k_A are significantly different, then using a normal calibration curve introduces a proportional determinate error.

Internal Standards

To use an external standardization or the method of standard additions, we must be able to treat identically all samples and standards. When this is not possible, the accuracy and precision of our standardization may suffer. For example, if our analyte is in a volatile solvent, then its concentration will increase if we lose solvent to evaporation. Suppose we have a sample and a standard with identical concentrations of analyte and identical signals. If both experience the same proportional loss of solvent, then their respective concentrations of analyte and signals remain identical. In effect, we can ignore evaporation if the samples and the standards experience an equivalent loss of solvent. If an identical standard and sample lose different amounts of solvent, however, then their respective concentrations and signals are no longer equal. In this case a simple external standardization or standard addition is not possible.

We can still complete a standardization if we reference the analyte's signal to a signal from another species that we add to all samples and standards. The species, which we call an ***internal standard***, must be different than the analyte.

Because the analyte and the internal standard receive the same treatment, the ratio of their signals is unaffected by any lack of reproducibility in the procedure. If a solution contains an analyte of concentration C_A and an internal standard of concentration C_{IS}, then the signals due to the analyte, S_A, and the internal standard, S_{IS}, are

$$S_A = k_A C_A$$

$$S_{IS} = k_{SI} C_{IS}$$

where k_A and k_{IS} are the sensitivities for the analyte and the internal standard, respectively. Taking the ratio of the two signals gives the fundamental equation for an internal standardization.

$$\frac{S_A}{S_{IS}} = \frac{k_A C_A}{k_{IS} C_{IS}} = K \times \frac{C_A}{C_{IS}} \tag{5.3.12}$$

Because K is a ratio of the analyte's sensitivity and the internal standard's sensitivity, it is not necessary to determine independently values for either k_A or k_{IS}.

Single Internal Standard

In a single-point internal standardization, we prepare a single standard that contains the analyte and the internal standard, and use it to determine the value of K in Equation 5.3.12.

$$K = \left(\frac{C_{IS}}{C_A}\right)_{std} \times \left(\frac{S_A}{S_{IS}}\right)_{std} \tag{5.3.13}$$

Having standardized the method, the analyte's concentration is given by

$$C_A = \frac{C_{IS}}{K} \times \left(\frac{S_A}{S_{IS}}\right)_{samp}$$

✔ Example 5.3.7

A sixth spectrophotometric method for the quantitative analysis of Pb^{2+} in blood uses Cu^{2+} as an internal standard. A standard that is 1.75 ppb Pb^{2+} and 2.25 ppb Cu^{2+} yields a ratio of $(S_A/S_{IS})_{std}$ of 2.37. A sample of blood spiked with the same concentration of Cu^{2+} gives a signal ratio, $(S_A/S_{IS})_{samp}$, of 1.80. What is the concentration of Pb^{2+} in the sample of blood?

Solution

Equation 5.3.13 allows us to calculate the value of K using the data for the standard

$$K = \left(\frac{C_{IS}}{C_A}\right)_{std} \times \left(\frac{S_A}{S_{IS}}\right)_{std} = \frac{2.25 \text{ ppb } Cu^{2+}}{1.75 \text{ ppb } Pb^{2+}} \times 2.37 = 3.05 \frac{\text{ppb } Cu^{2+}}{\text{ppb } Pb^{2+}}$$

The concentration of Pb^{2+}, therefore, is

$$C_A = \frac{C_{IS}}{K} \times \left(\frac{S_A}{S_{IS}}\right)_{samp} = \frac{2.25 \text{ ppb } Cu^{2+}}{3.05 \frac{\text{ppb } Cu^{2+}}{\text{ppb } Pb^{2+}}} \times 1.80 = 1.33 \text{ ppb } Pb^{2+}$$

Multiple Internal Standards

A single-point internal standardization has the same limitations as a single-point normal calibration. To construct an internal standard calibration curve we prepare a series of standards, each of which contains the same concentration of internal standard and a different concentrations of analyte. Under these conditions a calibration curve of $(S_A/S_{IS})_{std}$ versus C_A is linear with a slope of K/C_{IS}.

> Although the usual practice is to prepare the standards so that each contains an identical amount of the internal standard, this is not a requirement.

✔ Example 5.3.8

A seventh spectrophotometric method for the quantitative analysis of Pb^{2+} in blood gives a linear internal standards calibration curve for which

$$\left(\frac{S_A}{S_{IS}}\right)_{std} = (2.11 \text{ ppb}^{-1} \times C_A) - 0.006$$

What is the ppb Pb^{2+} in a sample of blood if (S_A/S_{IS})samp is 2.80?

Solution

To determine the concentration of Pb^{2+} in the sample of blood we replace $(S_A/S_{IS})_{std}$ in the calibration equation with $(S_A/S_{IS})_{samp}$ and solve for C_A.

$$C_A = \frac{\left(\frac{S_A}{S_{IS}}\right)_{samp} + 0.006}{2.11 \text{ ppb}^{-1}} = \frac{2.80 + 0.006}{2.11 \text{ ppb}^{-1}} = 1.33 \text{ ppb } Pb^{2+}$$

The concentration of Pb^{2+} in the sample of blood is 1.33 ppb.

In some circumstances it is not possible to prepare the standards so that each contains the same concentration of internal standard. This is the case, for example, when we prepare samples by mass instead of volume. We can still prepare a calibration curve, however, by plotting $(S_A/S_{IS})_{std}$ versus C_A/C_{IS}, giving a linear calibration curve with a slope of K.

> You might wonder if it is possible to include an internal standard in the method of standard additions to correct for both matrix effects and uncontrolled variations between samples; well, the answer is yes as described in the paper "Standard Dilution Analysis," the full reference for which is Jones, W. B.; Donati, G. L.; Calloway, C. P.; Jones, B. T. *Anal. Chem.* **2015**, *87*, 2321-2327.

5.4: Linear Regression and Calibration Curves

In a single-point external standardization we determine the value of k_A by measuring the signal for a single standard that contains a known concentration of analyte. Using this value of k_A and our sample's signal, we then calculate the concentration of analyte in our sample (see Example 5.3.1). With only a single determination of k_A, a quantitative analysis using a single-point external standardization is straightforward.

A multiple-point standardization presents a more difficult problem. Consider the data in Table 5.4.1 for a multiple-point external standardization. What is our best estimate of the relationship between S_{std} and C_{std}? It is tempting to treat this data as five separate single-point standardizations, determining k_A for each standard, and reporting the mean value for the five trials. Despite it simplicity, this is not an appropriate way to treat a multiple-point standardization.

Table 5.4.1 : Data for a Hypothetical Multiple-Point External Standardization

C_{std} (arbitrary units)	S_{std} (arbitrary units)	$k_A = S_{std}/C_{std}$
0.000	0.00	—
0.100	12.36	123.6
0.200	24.83	124.2
0.300	35.91	119.7
0.400	48.79	122.0
0.500	60.42	122.8
		mean k_A = 122.5

So why is it inappropriate to calculate an average value for k_A using the data in Table 5.4.1 ? In a single-point standardization we assume that the reagent blank (the first row in Table 5.4.1) corrects for all constant sources of determinate error. If this is not the case, then the value of k_A from a single-point standardization has a constant determinate error. Table 5.4.2 demonstrates how an uncorrected constant error affects our determination of k_A. The first three columns show the concentration of analyte in a set of standards, C_{std}, the signal without any source of constant error, S_{std}, and the actual value of k_A for five standards. As we expect, the value of k_A is the same for each standard. In the fourth column we add a constant determinate error of +0.50 to the signals, $(S_{std})_e$. The last column contains the corresponding apparent values of k_A. Note that we obtain a different value of k_A for each standard and that each apparent k_A is greater than the true value.

Table 5.4.2 : Effect of a Constant Determinate Error on the Value of k_A From a Single-Point Standardization

C_{std}	S_{std} (without constant error)	$k_A = S_{std}/C_{std}$ (actual)	$(S_{std})_e$ (with constant error)	$k_A = (S_{std})_e/C_{std}$ (apparent)
1.00	1.00	1.00	1.50	1.50
2.00	2.00	1.00	2.50	1.25
3.00	3.00	1.00	3.50	1.17
4.00	4.00	1.00	4.50	1.13
5.00	5.00	1.00	5.50	1.10
		mean k_A (true) = 1.00		mean k_A (apparent) = 1.23

How do we find the best estimate for the relationship between the signal and the concentration of analyte in a multiple-point standardization? Figure 5.4.1 shows the data in Table 5.4.1 plotted as a normal calibration curve. Although the data certainly appear to fall along a straight line, the actual calibration curve is not intuitively obvious. The process of determining the best equation for the calibration curve is called linear regression.

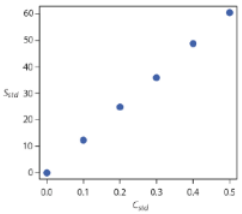


Figure 5.4.1 : Normal calibration curve data for the hypothetical multiple-point external standardization in Table 5.4.1 .

Linear Regression of Straight Line Calibration Curves

When a calibration curve is a straight-line, we represent it using the following mathematical equation

$$y = \beta_0 + \beta_1 x \tag{5.4.1}$$

where y is the analyte's signal, S_{std}, and x is the analyte's concentration, C_{std}. The constants β_0 and β_1 are, respectively, the calibration curve's expected y-intercept and its expected slope. Because of uncertainty in our measurements, the best we can do is to estimate values for β_0 and β_1, which we represent as b_0 and b_1. The goal of a ***linear regression*** analysis is to determine the best estimates for b_0 and b_1. How we do this depends on the uncertainty in our measurements.

Unweighted Linear Regression with Errors in *y*

The most common method for completing the linear regression for Equation 5.4.1 makes three assumptions:

1. that the difference between our experimental data and the calculated regression line is the result of indeterminate errors that affect y
2. that indeterminate errors that affect y are normally distributed
3. that the indeterminate errors in y are independent of the value of x

Because we assume that the indeterminate errors are the same for all standards, each standard contributes equally in our estimate of the slope and the y-intercept. For this reason the result is considered an ***unweighted linear regression***.

The second assumption generally is true because of the central limit theorem, which we considered in Chapter 4. The validity of the two remaining assumptions is less obvious and you should evaluate them before you accept the results of a linear regression. In particular the first assumption always is suspect because there certainly is some indeterminate error in the measurement of x. When we prepare a calibration curve, however, it is not unusual to find that the uncertainty in the signal, S_{std}, is significantly larger than the uncertainty in the analyte's concentration, C_{std}. In such circumstances the first assumption is usually reasonable.

How a Linear Regression Works

To understand the logic of a linear regression consider the example shown in Figure 5.4.2 , which shows three data points and two possible straight-lines that might reasonably explain the data. How do we decide how well these straight-lines fit the data, and how do we determine the best straight-line?

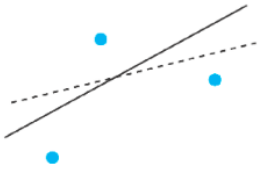


Figure 5.4.2 : Illustration showing three data points and two possible straight-lines that might explain the data. The goal of a linear regression is to find the mathematical model, in this case a straight-line, that best explains the data.

Let's focus on the solid line in Figure 5.4.2 . The equation for this line is

$$\hat{y} = b_0 + b_1 x \tag{5.4.2}$$

where b_0 and b_1 are estimates for the *y*-intercept and the slope, and $\hat{y}$ is the predicted value of *y* for any value of *x*. Because we assume that all uncertainty is the result of indeterminate errors in *y*, the difference between *y* and $\hat{y}$ for each value of *x* is the residual error, *r*, in our mathematical model.

$$r_i = (y_i - \hat{y}_i)$$

Figure 5.4.3 shows the residual errors for the three data points. The smaller the total residual error, R, which we define as

$$R = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \tag{5.4.3}$$

the better the fit between the straight-line and the data. In a linear regression analysis, we seek values of b_0 and b_1 that give the smallest total residual error.

> The reason for squaring the individual residual errors is to prevent a positive residual error from canceling out a negative residual error. You have seen this before in the equations for the sample and population standard deviations. You also can see from this equation why a linear regression is sometimes called the method of least squares.

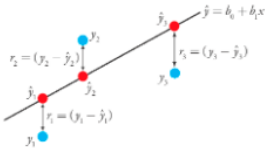


Figure 5.4.3 : Illustration showing the evaluation of a linear regression in which we assume that all uncertainty is the result of indeterminate errors in *y*. The points in blue, *y* , are the original data and the points in red, y_i , are the predicted values from the regression equation, $\hat{y} = b_0 + b_1 x$.The smaller the total residual error (Equation 5.4.3), the better the fit of the straight-line to the data.

Finding the Slope and *y*-Intercept

Although we will not formally develop the mathematical equations for a linear regression analysis, you can find the derivations in many standard statistical texts [See, for example, Draper, N. R.; Smith, H. Applied Regression Analysis, 3rd ed.; Wiley: New York, 1998]. The resulting equation for the slope, b_1, is

$$b_1 = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2} \tag{5.4.4}$$

and the equation for the *y*-intercept, b_0, is

$$b_0 = \frac{\sum_{i=1}^{n} y_i - b_1 \sum_{i=1}^{n} x_i}{n} \tag{5.4.5}$$

Although Equation 5.4.4 and Equation 5.4.5 appear formidable, it is necessary only to evaluate the following four summations

$$\sum_{i=1}^{n} x_i \quad \sum_{i=1}^{n} y_i \quad \sum_{i=1}^{n} x_i y_i \quad \sum_{i=1}^{n} x_i^2$$

Many calculators, spreadsheets, and other statistical software packages are capable of performing a linear regression analysis based on this model. To save time and to avoid tedious calculations, learn how to use one of these tools (and see Section 5.6 for details on completing a linear regression analysis using Excel and R.). For illustrative purposes the necessary calculations are shown in detail in the following example.

> Equation 5.4.4 and Equation 5.4.5 are written in terms of the general variables *x* and *y*. As you work through this example, remember that *x* corresponds to C_{std}, and that *y* corresponds to S_{std}.

✔ Example 5.4.1

Using the data from Table 5.4.1 , determine the relationship between S_{std} and C_{std} using an unweighted linear regression.

Solution

We begin by setting up a table to help us organize the calculation.

x_i	y_i	$x_i y_i$	x_i^2
0.000	0.00	0.000	0.000
0.100	12.36	1.236	0.010
0.200	24.83	4.966	0.040
0.300	35.91	10.773	0.090
0.400	48.79	19.516	0.160
0.500	60.42	30.210	0.250

Adding the values in each column gives

$$\sum_{i=1}^{n} x_i = 1.500 \quad \sum_{i=1}^{n} y_i = 182.31 \quad \sum_{i=1}^{n} x_i y_i = 66.701 \quad \sum_{i=1}^{n} x_i^2 = 0.550$$

Substituting these values into Equation 5.4.4 and Equation 5.4.5, we find that the slope and the *y*-intercept are

$$b_1 = \frac{(6 \times 66.701) - (1.500 \times 182.31)}{(6 \times 0.550) - (1.500)^2} = 120.706 \approx 120.71$$

$$b_0 = \frac{182.31 - (120.706 \times 1.500)}{6} = 0.209 \approx 0.21$$

The relationship between the signal and the analyte, therefore, is

$$S_{std} = 120.71 \times C_{std} + 0.21$$

For now we keep two decimal places to match the number of decimal places in the signal. The resulting calibration curve is shown in Figure 5.4.4 .

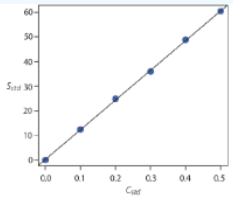


Figure 5.4.4 : Calibration curve for the data in Table 5.4.1 and Example 5.4.1 .

Uncertainty in the Regression Analysis

As shown in Figure 5.4.4 , because indeterminate errors in the signal, the regression line may not pass through the exact center of each data point. The cumulative deviation of our data from the regression line—that is, the total residual error—is proportional to the uncertainty in the regression. We call this uncertainty the ***standard deviation about the regression***, s_r, which is equal to

$$s_r = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n-2}} \tag{5.4.6}$$

where y_i is the i^{th} experimental value, and $\hat{y}_i$ is the corresponding value predicted by the regression line in Equation 5.4.2. Note that the denominator of Equation 5.4.6 indicates that our regression analysis has $n - 2$ degrees of freedom—we lose two degree of freedom because we use two parameters, the slope and the *y*-intercept, to calculate $\hat{y}_i$.

> Did you notice the similarity between the standard deviation about the regression (Equation 5.4.6) and the standard deviation for a sample (Equation 4.1.1)?

A more useful representation of the uncertainty in our regression analysis is to consider the effect of indeterminate errors on the slope, b_1, and the *y*-intercept, b_0, which we express as standard deviations.

$$s_{b_1} = \sqrt{\frac{n s_r^2}{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2}} = \sqrt{\frac{s_r^2}{\sum_{i=1}^{n} (x_i - \bar{x})^2}} \tag{5.4.7}$$

$$s_{b_0} = \sqrt{\frac{s_r^2 \sum_{i=1}^{n} x_i^2}{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2}} = \sqrt{\frac{s_r^2 \sum_{i=1}^{n} x_i^2}{n\sum_{i=1}^{n} (x_i - \bar{x})^2}} \tag{5.4.8}$$

We use these standard deviations to establish confidence intervals for the expected slope, β_1, and the expected *y*-intercept, β_0

$$\beta_1 = b_1 \pm t s_{b_1} \tag{5.4.9}$$

$$\beta_0 = b_0 \pm t s_{b_0} \tag{5.4.10}$$

where we select t for a significance level of α and for $n - 2$ degrees of freedom. Note that Equation 5.4.9 and Equation 5.4.10 do not contain a factor of $(\sqrt{n})^{-1}$ because the confidence interval is based on a single regression line.

Example 5.4.2

Calculate the 95% confidence intervals for the slope and *y*-intercept from Example 5.4.1 .

Solution

We begin by calculating the standard deviation about the regression. To do this we must calculate the predicted signals, $\hat{y}_i$, using the slope and *y*-intercept from Example 5.4.1 , and the squares of the residual error, $(y_i - \hat{y}_i)^2$. Using the last standard as an example, we find that the predicted signal is

$$\hat{y}_6 = b_0 + b_1 x_6 = 0.209 + (120.706 \times 0.500) = 60.562$$

and that the square of the residual error is

$$(y_i - \hat{y}_i)^2 = (60.42 - 60.562)^2 = 0.2016 \approx 0.202$$

The following table displays the results for all six solutions.

x_i	y_i	$\hat{y}_i$	$(y_i - \hat{y}_i)^2$
0.000	0.00	0.209	0.0437
0.100	12.36	12.280	0.0064
0.200	24.83	24.350	0.2304
0.300	35.91	36.421	0.2611
0.400	48.79	48.491	0.0894
0.500	60.42	60.562	0.0202

Adding together the data in the last column gives the numerator of Equation 5.4.6 as 0.6512; thus, the standard deviation about the regression is

$$s_r = \sqrt{\frac{0.6512}{6-2}} = 0.4035$$

Next we calculate the standard deviations for the slope and the *y*-intercept using Equation 5.4.7 and Equation 5.4.8. The values for the summation terms are from Example 5.4.1 .

$$s_{b_1} = \sqrt{\frac{6 \times (0.4035)^2}{(6 \times 0.550) - (1.500)^2}} = 0.965$$

$$s_{b_0} = \sqrt{\frac{(0.4035)^2 \times 0.550}{(6 \times 0.550) - (1.500)^2}} = 0.292$$

Finally, the 95% confidence intervals ($\alpha = 0.05$, 4 degrees of freedom) for the slope and *y*-intercept are

$$\beta_1 = b_1 \pm t s_{b_1} = 120.706 \pm (2.78 \times 0.965) = 120.7 \pm 2.7$$

$$\beta_0 = b_0 \pm t s_{b_0} = 0.209 \pm (2.78 \times 0.292) = 0.2 \pm 0.80$$

Finally, the 95% confidence intervals ($\alpha = 0.05$, 4 degrees of freedom) for the slope and *y*-intercept are

$$\beta_1 = b_1 \pm t s_{b_1} = 120.706 \pm (2.78 \times 0.965) = 120.7 \pm 2.7$$

$$\beta_0 = b_0 \pm t s_{b_0} = 0.209 \pm (2.78 \times 0.292) = 0.2 \pm 0.80$$

where *t*(0.05, 4) from Appendix 4 is 2.78. The standard deviation about the regression, s_r, suggests that the signal, S_{std}, is precise to one decimal place. For this reason we report the slope and the *y*-intercept to a single decimal place.

Minimizing Uncertainty in Calibration Model

To minimize the uncertainty in a calibration curve's slope and *y*-intercept, we evenly space our standards over a wide range of analyte concentrations. A close examination of Equation 5.4.7 and Equation 5.4.8 help us appreciate why this is true. The denominators of both equations include the term $\sum_{i=1}^{n}(x_i - \bar{x}_i)^2$. The larger the value of this term—which we accomplish by increasing the range of *x* around its mean value—the smaller the standard deviations in the slope and the *y*-intercept. Furthermore, to minimize the uncertainty in the *y*-intercept, it helps to decrease the value of the term $\sum_{i=1}^{n} x_i$ in Equation 5.4.8, which we accomplish by including standards for lower concentrations of the analyte.

Obtaining the Analyte's Concentration From a Regression Equation

Once we have our regression equation, it is easy to determine the concentration of analyte in a sample. When we use a normal calibration curve, for example, we measure the signal for our sample, S_{samp}, and calculate the analyte's concentration, C_A, using the regression equation.

$$C_A = \frac{S_{samp} - b_0}{b_1} \tag{5.4.11}$$

What is less obvious is how to report a confidence interval for C_A that expresses the uncertainty in our analysis. To calculate a confidence interval we need to know the standard deviation in the analyte's concentration, s_{C_A}, which is given by the following equation

$$s_{C_A} = \frac{s_r}{b_1}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\left(\bar{S}_{samp} - \bar{S}_{std}\right)^2}{(b_1)^2 \sum_{i=1}^{n}\left(C_{std_i} - \bar{C}_{std}\right)^2}} \tag{5.4.12}$$

where m is the number of replicate we use to establish the sample's average signal, S_{samp}, n is the number of calibration standards, S_{std} is the average signal for the calibration standards, and C_{std_i} and $\overline{C}_{std}$ are the individual and the mean concentrations for the calibration standards. Knowing the value of s_{C_A}, the confidence interval for the analyte's concentration is

$$\mu_{C_A} = C_A \pm t s_{C_A}$$

where μ_{C_A} is the expected value of C_A in the absence of determinate errors, and with the value of t is based on the desired level of confidence and $n - 2$ degrees of freedom.

Equation 5.4.12 is written in terms of a calibration experiment. A more general form of the equation, written in terms of x and y, is given here.

$$s_x = \frac{s_r}{b_1}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\left(\overline{Y} - \overline{y}\right)^2}{(b_1)^2 \sum_{i=1}^{n}(x_i - \overline{x})^2}}$$

A close examination of Equation 5.4.12 should convince you that the uncertainty in C_A is smallest when the sample's average signal, $\overline{S}_{samp}$, is equal to the average signal for the standards, $\overline{S}_{std}$. When practical, you should plan your calibration curve so that S_{samp} falls in the middle of the calibration curve. For more information about these regression equations see (a) Miller, J. N. *Analyst* **1991**, *116*, 3–14; (b) Sharaf, M. A.; Illman, D. L.; Kowalski, B. R. *Chemometrics*, Wiley-Interscience: New York, 1986, pp. 126-127; (c) Analytical Methods Committee "Uncertainties in concentrations estimated from calibration experiments," AMC Technical Brief, March 2006.

Example 5.4.3

Three replicate analyses for a sample that contains an unknown concentration of analyte, yield values for S_{samp} of 29.32, 29.16 and 29.51 (arbitrary units). Using the results from Example 5.4.1 and Example 5.4.2 , determine the analyte's concentration, C_A, and its 95% confidence interval.

Solution

The average signal, $\overline{S}_{samp}$, is 29.33, which, using Equation 5.4.11 and the slope and the y-intercept from Example 5.4.1 , gives the analyte's concentration as

$$C_A = \frac{\overline{S}_{samp} - b_0}{b_1} = \frac{29.33 - 0.209}{120.706} = 0.241$$

To calculate the standard deviation for the analyte's concentration we must determine the values for $\overline{S}_{std}$ and for $\sum_{i=1}^{2}(C_{std_i} - \overline{C}_{std})^2$. The former is just the average signal for the calibration standards, which, using the data in Table 5.4.1 , is 30.385. Calculating $\sum_{i=1}^{2}(C_{std_i} - \overline{C}_{std})^2$ looks formidable, but we can simplify its calculation by recognizing that this sum-of-squares is the numerator in a standard deviation equation; thus,

$$\sum_{i=1}^{n}(C_{std_i} - \overline{C}_{std})^2 = (s_{C_{std}})^2 \times (n-1)$$

where $s_{C_{std}}$ is the standard deviation for the concentration of analyte in the calibration standards. Using the data in Table 5.4.1 we find that $s_{C_{std}}$ is 0.1871 and

$$\sum_{i=1}^{n}(C_{std_i} - \overline{C}_{std})^2 = (0.1872)^2 \times (6-1) = 0.175$$

Substituting known values into Equation 5.4.12 gives

$$s_{C_A} = \frac{0.4035}{120.706}\sqrt{\frac{1}{3} + \frac{1}{6} + \frac{(29.33 - 30.385)^2}{(120.706)^2 \times 0.175}} = 0.0024$$

Finally, the 95% confidence interval for 4 degrees of freedom is

$$\mu_{C_A} = C_A \pm t s_{C_A} = 0.241 \pm (2.78 \times 0.0024) = 0.241 \pm 0.007$$

Figure 5.4.5 shows the calibration curve with curves showing the 95% confidence interval for C_A.

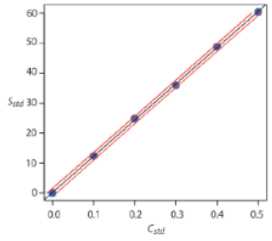


Figure 5.4.5 : Example of a normal calibration curve with a superimposed confidence interval for the analyte's concentration. The points in blue are the original data from Table 5.4.1 . The black line is the normal calibration curve as determined in Example 5.4.1 . The red lines show the 95% confidence interval for C_A assuming a single determination of S_{samp}.

In a standard addition we determine the analyte's concentration by extrapolating the calibration curve to the x-intercept. In this case the value of C_A is

$$C_A = x\text{-intercept} = \frac{-b_0}{b_1}$$

and the standard deviation in C_A is

$$s_{C_A} = \frac{s_r}{b_1}\sqrt{\frac{1}{n} + \frac{(\overline{S}_{std})^2}{(b_1)^2 \sum_{i=1}^{n}(C_{std_i} - \overline{C}_{std})^2}}$$

where n is the number of standard additions (including the sample with no added standard), and S_{std} is the average signal for the n standards. Because we determine the analyte's concentration by extrapolation, rather than by interpolation, s_{C_A} for the method of standard additions generally is larger than for a normal calibration curve.

? Exercise 5.4.1

Figure 5.4.2 shows a normal calibration curve for the quantitative analysis of Cu^{2+}. The data for the calibration curve are shown here.

$[Cu^{2+}]$ (M)	Absorbance
0	0
1.55×10^{-3}	0.050
3.16×10^{-3}	0.093
4.74×10^{-3}	0.143
6.34×10^{-3}	0.188
7.92×10^{-3}	0.236

Complete a linear regression analysis for this calibration data, reporting the calibration equation and the 95% confidence interval for the slope and the y-intercept. If three replicate samples give an S_{samp} of 0.114, what is the concentration of analyte in the sample and its 95% confidence interval?

Answer

We begin by setting up a table to help us organize the calculation

x_i	y_i	$x_i y_i$	x_i^2
0.000	0.000	0.000	0.000
1.55×10^{-3}	0.050	7.750×10^{-5}	2.403×10^{-6}
3.16×10^{-3}	0.093	2.939×10^{-4}	9.986×10^{-6}
4.74×10^{-3}	0.143	6.778×10^{-4}	2.247×10^{-5}
6.34×10^{-3}	0.188	1.192×10^{-3}	4.020×10^{-5}
7.92×10^{-3}	0.236	1.869×10^{-3}	6.273×10^{-5}

Adding the values in each column gives

$$\sum_{i=1}^{n} x_i = 2.371 \times 10^{-2} \quad \sum_{i=1}^{n} y_i = 0.710 \quad \sum_{i=1}^{n} x_i y_i = 4.110 \times 10^{-3} \quad \sum_{i=1}^{n} x_i^2 = 1.378 \times 10^{-4}$$

When we substitute these values into Equation 5.4.4 and Equation 5.4.5, we find that the slope and the y-intercept are

$$b_1 = \frac{6 \times (4.110 \times 10^{-3}) - (2.371 \times 10^{-2}) \times 0.710}{6 \times (1.378 \times 10^{-4}) - (2.371 \times 10^{-2})^2}) = 29.57$$

$$b_0 = \frac{0.710 - 29.57 \times (2.371 \times 10^{-2}}{6} = 0.0015$$

and that the regression equation is

$$S_{std} = 29.57 \times C_{std} + 0.0015$$

To calculate the 95% confidence intervals, we first need to determine the standard deviation about the regression. The following table helps us organize the calculation.

x_i	y_i	$\hat{y}_i$	$(y_i - \hat{y}_i)^2$
0.000	0.000	0.0015	2.250×10^{-6}
1.55×10^{-3}	0.050	0.0473	7.110×10^{-6}
3.16×10^{-3}	0.093	0.0949	3.768×10^{-6}
4.74×10^{-3}	0.143	0.1417	1.791×10^{-6}
6.34×10^{-3}	0.188	0.1890	9.483×10^{-6}
7.92×10^{-3}	0.236	0.2357	9.339×10^{-6}

Adding together the data in the last column gives the numerator of Equation 5.4.6 as 1.596×10^{-5}. The standard deviation about the regression, therefore, is

$$s_r = \sqrt{\frac{1.596 \times 10^{-5}}{6 - 2}} = 1.997 \times 10^{-3}$$

Next, we need to calculate the standard deviations for the slope and the y-intercept using Equation 5.4.7 and Equation 5.4.8.

$$s_{b_1} = \sqrt{\frac{6 \times (1.997 \times 10^{-3})^2}{6 \times (1.378 \times 10^{-4}) - (2.371 \times 10^{-2})^2}} = 0.3007$$

$$s_{b_0} = \sqrt{\frac{(1.997 \times 10^{-3})^2 \times (1.378 \times 10^{-4})}{6 \times (1.378 \times 10^{-4}) - (2.371 \times 10^{-2})^2}} = 1.441 \times 10^{-3}$$

and use them to calculate the 95% confidence intervals for the slope and the *y*-intercept

$$\beta_1 = b_1 \pm ts_{b_1} = 29.57 \pm (2.78 \times 0.3007) = 29.57\ \text{M}^{-1} \pm 0.84\ \text{M}^{-1}$$

$$\beta_0 = b_0 \pm ts_{b_0} = 0.0015 \pm (2.78 \times 1.441 \times 10^{-3}) = 0.0015 \pm 0.0040$$

With an average S_{samp} of 0.114, the concentration of analyte, C_A, is

$$C_A = \frac{S_{samp} - b_0}{b_1} = \frac{0.114 - 0.0015}{29.57\ \text{M}^{-1}} = 3.80 \times 10^{-3}\ \text{M}$$

The standard deviation in C_A is

$$s_{C_A} = \frac{1.997 \times 10^{-3}}{29.57} \sqrt{\frac{1}{3} + \frac{1}{6} + \frac{(0.114 - 0.1183)^2}{(29.57)^2 \times (4.408 \times 10^{-5})}} = 4.778 \times 10^{-5}$$

and the 95% confidence interval is

$$\mu = C_A \pm ts_{C_A} = 3.80 \times 10^{-3} \pm \{2.78 \times (4.778 \times 10^{-5})\}$$

$$\mu = 3.80 \times 10^{-3}\ \text{M} \pm 0.13 \times 10^{-3}\ \text{M}$$

Evaluating a Linear Regression Model

You should never accept the result of a linear regression analysis without evaluating the validity of the model. Perhaps the simplest way to evaluate a regression analysis is to examine the residual errors. As we saw earlier, the residual error for a single calibration standard, r_i, is

$$r_i = (y_i - \hat{y}_i)$$

If the regression model is valid, then the residual errors should be distributed randomly about an average residual error of zero, with no apparent trend toward either smaller or larger residual errors (Figure 5.4.6 a). Trends such as those in Figure 5.4.6 b and Figure 5.4.6 c provide evidence that at least one of the model's assumptions is incorrect. For example, a trend toward larger residual errors at higher concentrations, Figure 5.4.6 b, suggests that the indeterminate errors affecting the signal are not independent of the analyte's concentration. In Figure 5.4.6 c, the residual errors are not random, which suggests we cannot model the data using a straight-line relationship. Regression methods for the latter two cases are discussed in the following sections.

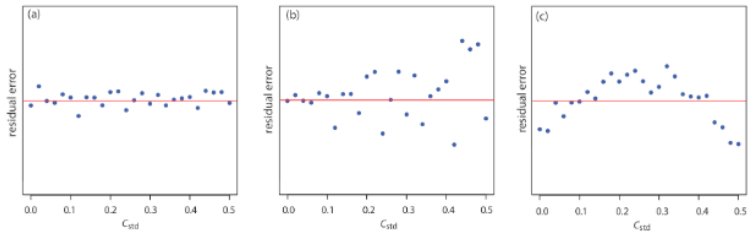


Figure 5.4.6 : Plots of the residual error in the signal, S_{std}, as a function of the concentration of analyte, C_{std}, for an unweighted straight-line regression model. The red line shows a residual error of zero. The distribution of the residual errors in (a) indicates that the unweighted linear regression model is appropriate. The increase in the residual errors in (b) for higher concentrations of analyte, suggests that a weighted straight-line regression is more appropriate. For (c), the curved pattern to the residuals suggests that a straight-line model is inappropriate; linear regression using a quadratic model might produce a better fit.

? Exercise 5.4.2

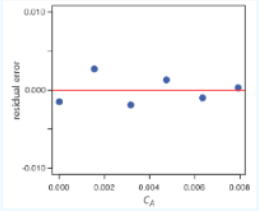
Using your results from Exercise 5.4.1 , construct a residual plot and explain its significance.

Answer

To create a residual plot, we need to calculate the residual error for each standard. The following table contains the relevant information.

x_i	y_i	$\hat{y}_i$	$y_i - \hat{y}_i$
0.000	0.000	0.0015	–0.0015
1.55×10^{-3}	0.050	0.0473	0.0027
3.16×10^{-3}	0.093	0.0949	–0.0019
4.74×10^{-3}	0.143	0.1417	0.0013
6.34×10^{-3}	0.188	0.1890	–0.0010
7.92×10^{-3}	0.236	0.2357	0.0003

The figure below shows a plot of the resulting residual errors. The residual errors appear random, although they do alternate in sign, and that do not show any significant dependence on the analyte's concentration. Taken together, these observations suggest that our regression model is appropriate.



Weighted Linear Regression with Errors in *y*

Our treatment of linear regression to this point assumes that indeterminate errors affecting *y* are independent of the value of *x*. If this assumption is false, as is the case for the data in Figure 5.4.6 b, then we must include the variance for each value of *y* into our determination of the *y*-intercept, b_0, and the slope, b_1; thus

$$b_0 = \frac{\sum_{i=1}^{n} w_i y_i - b_1 \sum_{i=1}^{n} w_i x_i}{n} \tag{5.4.13}$$

$$b_1 = \frac{n\sum_{i=1}^{n} w_i x_i y_i - \sum_{i=1}^{n} w_i x_i \sum_{i=1}^{n} w_i y_i}{n\sum_{i=1}^{n} w_i x_i^2 - \left(\sum_{i=1}^{n} w_i x_i\right)^2} \tag{5.4.14}$$

where w_i is a weighting factor that accounts for the variance in y_i

$$w_i = \frac{n(s_{y_i})^{-2}}{\sum_{i=1}^{n}(s_{y_i})^{-2}} \tag{5.4.15}$$

and s_{y_i} is the standard deviation for y_i. In a ***weighted linear regression***, each *xy*-pair's contribution to the regression line is inversely proportional to the precision of y_i; that is, the more precise the value of *y*, the greater its contribution to the regression.

Example 5.4.4

Shown here are data for an external standardization in which *sstd* is the standard deviation for three replicate determination of the signal. This is the same data used in Example 5.4.1 with additional information about the standard deviations in the signal.

C_{std} (arbitrary units)	S_{std} (arbitrary units)	s_{std}
0.000	0.00	0.02
0.100	12.36	0.02
0.200	24.83	0.07
0.300	35.91	0.13
0.400	48.79	0.22
0.400	48.79	0.22
0.500	60.42	0.33

Determine the calibration curve's equation using a weighted linear regression. As you work through this example, remember that *x* corresponds to C_{std}, and that *y* corresponds to S_{std}.

Solution

We begin by setting up a table to aid in calculating the weighting factors.

C_{std} (arbitrary units)	S_{std} (arbitrary units)	s_{std}	$(s_{y_i})^{-2}$	w_i
0.000	0.00	0.02	2500.00	2.8339
0.100	12.36	0.02	250.00	2.8339
0.200	24.83	0.07	204.08	0.2313
0.300	35.91	0.13	59.17	0.0671
0.400	48.79	0.22	20.66	0.0234
0.500	60.42	0.33	9.18	0.0104

Adding together the values in the fourth column gives

$$\sum_{i=1}^{n}(s_{y_i})^{-2}$$

which we use to calculate the individual weights in the last column. As a check on your calculations, the sum of the individual weights must equal the number of calibration standards, *n*. The sum of the entries in the last column is 6.0000, so all is well. After we calculate the individual weights, we use a second table to aid in calculating the four summation terms in Equation 5.4.13 and Equation 5.4.14.

x_i	y_i	w_i	$w_i x_i$	$w_i y_i$	$w_i x_i^2$	$w_i x_i y_i$
0.000	0.00	2.8339	0.0000	0.0000	0.0000	0.0000
0.100	12.36	2.8339	0.2834	35.0270	0.0283	3.5027
0.200	24.83	0.2313	0.0463	5.7432	0.0093	1.1486
0.300	35.91	0.0671	0.0201	2.4096	0.0060	0.7229
0.400	48.79	0.0234	0.0094	1.1417	0.0037	0.4567
0.500	60.42	0.0104	0.0052	0.6284	0.0026	0.3142

Adding the values in the last four columns gives

$$\sum_{i=1}^{n} w_i x_i = 0.3644 \quad \sum_{i=1}^{n} w_i y_i = 44.9499 \quad \sum_{i=1}^{n} w_i x_i^2 = 0.0499 \quad \sum_{i=1}^{n} w_i x_i y_i = 6.1451$$

Substituting these values into the Equation 5.4.13 and Equation 5.4.14 gives the estimated slope and estimated *y*-intercept as

$$b_1 = \frac{(6 \times 6.1451) - (0.3644 \times 44.9499)}{(6 \times 0.0499) - (0.3644)^2} = 122.985$$

$$b_0 = \frac{44.9499 - (122.985 \times 0.3644)}{6} = 0.0224$$

The calibration equation is

$$S_{std} = 122.98 \times C_{std} + 0.2$$

Figure 5.4.7 shows the calibration curve for the weighted regression and the calibration curve for the unweighted regression in Example 5.4.1 . Although the two calibration curves are very similar, there are slight differences in the slope and in the *y*-intercept. Most notably, the *y*-intercept for the weighted linear regression is closer to the expected value of zero. Because the standard deviation for the signal, S_{std}, is smaller for smaller concentrations of analyte, C_{std}, a weighted linear regression gives more emphasis to these standards, allowing for a better estimate of the *y*-intercept.

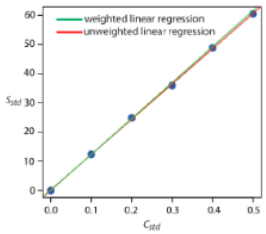


Figure 5.4.7 : A comparison of the unweighted and the weighted normal calibration curves. See Example 5.4.1 for details of the unweighted linear regression and Example 5.4.4 for details of the weighted linear regression.

Equations for calculating confidence intervals for the slope, the *y*-intercept, and the concentration of analyte when using a weighted linear regression are not as easy to define as for an unweighted linear regression [Bonate, P. J. *Anal. Chem.* **1993**, *65*, 1367–1372]. The confidence interval for the analyte's concentration, however, is at its optimum value when the analyte's signal is near the weighted centroid, y_c , of the calibration curve.

$$y_c = \frac{1}{n} \sum_{i=1}^{n} w_i x_i$$

Weighted Linear Regression with Errors in Both *x* and *y*

If we remove our assumption that indeterminate errors affecting a calibration curve are present only in the signal (*y*), then we also must factor into the regression model the indeterminate errors that affect the analyte's concentration in the calibration standards (*x*). The solution for the resulting regression line is computationally more involved than that for either the unweighted or weighted regression lines. Although we will not consider the details in this textbook, you should be aware that neglecting the presence of indeterminate errors in *x* can bias the results of a linear regression.

> See, for example, Analytical Methods Committee, "Fitting a linear functional relationship to data with error on both variable," AMC Technical Brief, March, 2002), as well as this chapter's Additional Resources.

Curvilinear and Multivariate Regression

A straight-line regression model, despite its apparent complexity, is the simplest functional relationship between two variables. What do we do if our calibration curve is curvilinear—that is, if it is a curved-line instead of a straight-line? One approach is to try transforming the data into a straight-line. Logarithms, exponentials, reciprocals, square roots, and trigonometric functions have been used in this way. A plot of log(*y*) versus *x* is a typical example. Such transformations are not without complications, of which the most obvious is that data with a uniform variance in *y* will not maintain that uniform variance after it is transformed.

> It is worth noting that the term "linear" does not mean a straight-line. A linear function may contain more than one additive term, but each such term has one and only one adjustable multiplicative parameter. The function
>
> $$y = ax + bx^2$$
>
> is an example of a linear function because the terms *x* and x^2 each include a single multiplicative parameter, *a* and *b*, respectively. The function
>
> $$y = x^b$$
>
> is nonlinear because *b* is not a multiplicative parameter; it is, instead, a power. This is why you can use linear regression to fit a polynomial equation to your data.
>
> Sometimes it is possible to transform a nonlinear function into a linear function. For example, taking the log of both sides of the nonlinear function above gives a linear function.
>
> $$\log(y) = b \log(x)$$

Another approach to developing a linear regression model is to fit a polynomial equation to the data, such as $y = a + bx + cx^2$. You can use linear regression to calculate the parameters *a*, *b*, and *c*, although the equations are different than those for the linear regression of a straight-line. If you cannot fit your data using a single polynomial equation, it may be possible to fit separate polynomial equations to short segments of the calibration curve. The result is a single continuous calibration curve known as a spline function.

> For details about curvilinear regression, see (a) Sharaf, M. A.; Illman, D. L.; Kowalski, B. R. *Chemometrics*, Wiley-Interscience: New York, 1986; (b) Deming, S. N.; Morgan, S. L. *Experimental Design: A Chemometric Approach*, Elsevier: Amsterdam, 1987.

The regression models in this chapter apply only to functions that contain a single independent variable, such as a signal that depends upon the analyte's concentration. In the presence of an interferent, however, the signal may depend on the concentrations of both the analyte and the interferent

$$S = k_A C_A + k_I C I + S_{reag}$$

where k_I is the interferent's sensitivity and C_I is the interferent's concentration. Multivariate calibration curves are prepared using standards that contain known amounts of both the analyte and the interferent, and modeled using multivariate regression.

> See Beebe, K. R.; Kowalski, B. R. *Anal. Chem.* **1987**, *59*, 1007A–1017A. for additional details, and check out this chapter's Additional Resources for more information about linear regression with errors in both variables, curvilinear regression, and multivariate regression.

5.5: Compensating for the Reagent Blank

Thus far in our discussion of strategies for standardizing analytical methods, we have assumed that a suitable reagent blank is available to correct for signals arising from sources other than the analyte. We did not, however ask an important question: "What constitutes an appropriate reagent blank?" Surprisingly, the answer is not immediately obvious.

In one study, approximately 200 analytical chemists were asked to evaluate a data set consisting of a normal calibration curve, a separate analyte-free blank, and three samples with different sizes, but drawn from the same source [Cardone, M. J. *Anal. Chem.* **1986**, *58*, 433–438]. The first two columns in Table 5.5.1 shows a series of external standards and their corresponding signals. The normal calibration curve for the data is

$$S_{std} = 0.0750 \times W_{std} + 0.1250$$

where the *y*-intercept of 0.1250 is the calibration blank. A separate reagent blank gives the signal for an analyte-free sample.

Table 5.5.1 : Data Used to Study the Blank in an Analytical Method

W_{std}	S_{std}	Sample Number	W_{samp}	S_{samp}
1.6667	0.2500	1	62.4746	0.8000
5.0000	0.5000	2	82.7915	1.0000
8.3333	0.7500	3	103.1085	1.2000
11.6667	0.8413			
18.1600	1.4870		reagent blank	0.1000
19.9333	1.6200			

$$S_{std} = 0.0750 \times W_{std} + 0.1250$$

W_{std}: weight of analyte used to prepare the external standard; diluted to a volume, V

W_{samp}: weight of sample used to prepare sample as analyzed; diluted to a volume, V

In working up this data, the analytical chemists used at least four different approaches to correct the signals: (a) ignoring both the calibration blank, *CB*, and the reagent blank, *RB*, which clearly is incorrect; (b) using the calibration blank only; (c) using the reagent blank only; and (d) using both the calibration blank and the reagent blank. The first four rows of Table 5.5.2 shows the equations for calculating the analyte's concentration using each approach, along with the reported concentrations for the analyte in each sample.

Table 5.5.2 : Equations and Resulting Concentrations of Analyte for Different Approaches to Correcting for the Blank

		Concentration of Analyte in...		
Approach for Correcting the Signal	Equation	Sample 1	Sample 2	Sample 3
ignore calibration and reagent blanks	$C_A = \frac{W_A}{W_{samp}} = \frac{S_{samp}}{k_A W_{samp}}$	0.1707	0.1610	0.1552
use calibration blank only	$C_A = \frac{W_A}{W_{samp}} = \frac{S_{samp} - CB}{k_A W_{samp}}$	0.1441	0.1409	0.1390
use reagent blank only	$C_A = \frac{W_A}{W_{samp}} = \frac{S_{samp} - RB}{k_A W_{samp}}$	0.1494	0.1449	0.1422
use both calibration and reagent blanks	$C_A = \frac{W_A}{W_{samp}} = \frac{S_{samp} - CB - RB}{k_A W_{samp}}$	0.1227	0.1248	0.1266
use total Youden blank	$C_A = \frac{W_A}{W_{samp}} = \frac{S_{samp} - TYB}{k_A W_{samp}}$	0.1313	0.1313	0.1313

$C_A =$ concentration of analyte; $W_A =$ weight of analyte; W_{samp} weight of sample;

$k_A =$ slope of calibration curve (0.0750; slope of calibration equation); $CB =$ calibration blank (0.125; intercept of calibration equation);

$RB =$ reagent blank (0.100); $TYB =$ total Youden blank (0.185; see text)

That all four methods give a different result for the analyte's concentration underscores the importance of choosing a proper blank, but does not tell us which blank is correct. Because all four methods fail to predict the same concentration of analyte for each sample, none of these blank corrections properly accounts for an underlying constant source of determinate error.

To correct for a constant method error, a blank must account for signals from any reagents and solvents used in the analysis and any bias that results from interactions between the analyte and the sample's matrix. Both the calibration blank and the reagent blank compensate for signals from reagents and solvents. Any difference in their values is due to indeterminate errors in preparing and analyzing the standards.

Unfortunately, neither a calibration blank nor a reagent blank can correct for a bias that results from an interaction between the analyte and the sample's matrix. To be effective, the blank must include both the sample's matrix and the analyte and, consequently, it must be determined using the sample itself. One approach is to measure the signal for samples of different size, and to determine the regression line for a plot of S_{samp} versus the amount of sample. The resulting *y*-intercept gives the signal in the absence of sample, and is known as the ***total Youden blank*** [Cardone, M. J. *Anal. Chem.* **1986**, *58*, 438–445]. This is the true blank correction. The regression line for the three samples in Table 5.5.1 is

$$S_{samp} = 0.009844 \times W_{samp} + 0.185$$

giving a true blank correction of 0.185. As shown in Table 5.5.2 , using this value to correct S_{samp} gives identical values for the concentration of analyte in all three samples.

The use of the total Youden blank is not common in analytical work, with most chemists relying on a calibration blank when using a calibration curve and a reagent blank when using a single-point standardization. As long we can ignore any constant bias due to interactions between the analyte and the sample's matrix, which is often the case, the accuracy of an analytical method will not suffer. It is a good idea, however, to check for constant sources of error before relying on either a calibration blank or a reagent blank.

Summary

In a quantitative analysis we measure a signal, S_{total}, and calculate the amount of analyte, n_A or C_A, using one of the following equations.

$$S_{total} = k_A n_A + S_{reag}$$

$$S_{total} = k_A C_A + S_{reag}$$

To obtain an accurate result we must eliminate determinate errors that affect the signal, S_{total}, the method's sensitivity, k_A, and the signal due to the reagents, S_{reag}.

To ensure that we accurately measure S_{total}, we calibrate our equipment and instruments. To calibrate a balance, for example, we use a standard weight of known mass. The manufacturer of an instrument usually suggests appropriate calibration standards and calibration methods.

To standardize an analytical method we determine its sensitivity. There are several standardization strategies available to us, including external standards, the method of standard addition, and internal standards. The most common strategy is a multiple-point external standardization and a normal calibration curve. We use the method of standard additions, in which we add known amounts of analyte to the sample, when the sample's matrix complicates the analysis. When it is difficult to reproducibly handle samples and standards, we may choose to add an internal standard.

Single-point standardizations are common, but are subject to greater uncertainty. Whenever possible, a multiple-point standardization is preferred, with results displayed as a calibration curve. A linear regression analysis provides an equation for the standardization.

A reagent blank corrects for any contribution to the signal from the reagents used in the analysis. The most common reagent blank is one in which an analyte-free sample is taken through the analysis. When a simple reagent blank does not compensate for all constant sources of determinate error, other types of blanks, such as the total Youden blank, are used.

6: Equilibrium Chemistry

Regardless of the problem on which an analytical chemist is working, its solution requires a knowledge of chemistry and the ability to apply that knowledge to solve a problem. For example, an analytical chemist who is studying the effect of pollution on spruce trees needs to know, or know where to find, the chemical differences between *p*-hydroxybenzoic acid and *p*-hydroxyacetophenone, two common phenols found in the needles of spruce trees.

The ability to "think as a chemist" is a product of your experience in the classroom and in the laboratory. For the most part, the material in this text assumes you are familiar with topics covered in earlier courses; however, because of its importance to analytical chemistry, this chapter provides a review of equilibrium chemistry. Much of the material in this chapter should be familiar to you, although some topics—ladder diagrams and activity, for example—likely afford you with new ways to look at equilibrium chemistry.

In 1798, the chemist Claude Berthollet accompanied Napoleon's military expedition to Egypt. While visiting the Natron Lakes, a series of salt water lakes carved from limestone, Berthollet made an observation that led him to an important discovery. When exploring the lake's shore, Berthollet found deposits of Na_2CO_3, a result he found surprising. Why did Berthollet find this result surprising and how did it contribute to an important discovery? Answering these questions provides us with an example of chemical reasoning and introduces us to the topic of this chapter.

At the end of the 18th century, chemical reactivity was explained in terms of elective affinities [Quilez, J. *Chem. Educ. Res. Pract.* **2004**, *5*, 69–87]. If, for example, substance A reacts with substance BC to form AB

$$\mathrm{A + BC \rightarrow AB + C}$$

then A and B were said to have an elective affinity for each other. With elective affinity as the driving force for chemical reactivity, reactions were understood to proceed to completion and to proceed in one direction. Once formed, the compound AB could not revert to A and BC.

$$\mathrm{A + BC \nrightarrow AB + C}$$

From his experience in the laboratory, Berthollet knew that adding solid Na_2CO_3 to a solution of $CaCl_2$ produces a precipitate of $CaCO_3$.

$$Na_2CO_3(s) + CaCl_2(aq) \rightarrow 2NaCl(aq) + CaCO_3(s)$$

Understanding this, Berthollet was surprised to find solid Na_2CO_3 forming on the edges of the lake, particularly since the deposits formed only when the lake's salt water, $NaCl(aq)$, was in contact with solid limestone, $CaCO_3(s)$. Where the lake was in contact with clay soils, there was little or no Na_2CO_3.

> Natron is another name for the mineral sodium carbonate, $Na_2CO_3 \bullet 10H_2O$. In nature, it usually contains impurities of $NaHCO_3$ and NaCl. In ancient Egypt, natron was mined and used for a variety of purposes, including as a cleaning agent and in mummification.

Berthollet's important insight was recognizing that the chemistry leading to the formation of Na_2CO_3 is the reverse of that seen in the laboratory.

$$2NaCl(aq) + CaCO_3(s) \rightarrow Na_2CO_3(s) + CaCl_2(aq)$$

Using this insight Berthollet reasoned that the reaction is reversible, and that the relative amounts of NaCl, $CaCO_3$, Na_2CO_3, and $CaCl_2$ determine the direction in which the reaction occurs and the final composition of the reaction mixture. We recognize a reaction's ability to move in both directions by using a double arrow when we write the reaction.

$$Na_2CO_3(s) + CaCl_2(aq) \rightleftharpoons 2NaCl(aq) + CaCO_3(s)$$

> For obvious reasons, we call the double arrow, $\rightleftharpoons$, an equilibrium arrow.

Berthollet's reasoning that reactions are reversible was an important step in understanding chemical reactivity. When we mix together solutions of Na_2CO_3 and $CaCl_2$ they react to produce NaCl and $CaCO_3$. As the reaction takes place, if we monitor the mass of Ca^{2+} that remains in solution and the mass of $CaCO_3$ that precipitates, the result looks something like Figure 6.1.1 . At the start of the reaction the mass of Ca^{2+} decreases and the mass of $CaCO_3$ increases. Eventually the reaction reaches a point after which there is no further change in the amounts of these species. Such a condition is called a state of ***equilibrium***.

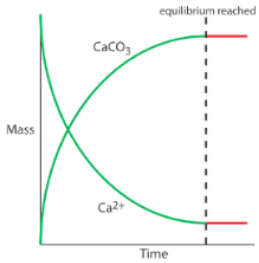


Figure 6.1.1 . Graph showing how the masses of Ca^{2+} and $CaCO_3$ change as a function of time during the precipitation of $CaCO_3$. The dashed line indicates when the reaction reaches equilibrium. Prior to equilibrium the masses of Ca^{2+} and $CaCO_3$ are changing; after equilibrium is reached, their masses remain constant.

Although a system at equilibrium appears static on a macroscopic level, it is important to remember that the forward and the reverse reactions continue to occur. A reaction at equilibrium exists in a ***steady-state***, in which the rate at which a species forms equals the rate at which it is consumed.

6.2: Thermodynamics and Equilibrium Chemistry

Thermodynamics is the study of thermal, electrical, chemical, and mechanical forms of energy. The study of thermodynamics crosses many disciplines, including physics, engineering, and chemistry. Of the various branches of thermodynamics, the most important to chemistry is the study of how energy changes during a chemical reaction.

Consider, for example, the general equilibrium reaction shown in Equation 6.2.1, which involves the species A, B, C, and D, with stoichiometric coefficients of *a*, *b*, *c*, and *d*.

$$aA + bB \rightleftharpoons cC + dD \tag{6.2.1}$$

By convention, we identify the species on the left side of the equilibrium arrow as reactants and those on the right side of the equilibrium arrow as products. As Berthollet discovered, writing a reaction in this fashion does not guarantee that the reaction of A and B to produce C and D is favorable. Depending on initial conditions the reaction may move to the left, it may move to the right, or it may exist in a state of equilibrium. Understanding the factors that determine the reaction's final equilibrium position is one of the goals of chemical thermodynamics.

The direction of a reaction is that which lowers the overall free energy. At a constant temperature and pressure, which is typical of many benchtop chemical reactions, a reaction's free energy is given by the ***Gibb's free energy*** function

$$\Delta G = \Delta H - T\Delta S \tag{6.2.2}$$

where T is the temperature in kelvin, and ΔG, ΔH, and ΔS are the differences in the Gibb's free energy, the enthalpy, and the entropy between the products and the reactants.

Enthalpy is a measure of the flow of energy, as heat, during a chemical reaction. A reaction that releases heat has a negative ΔH and is called exothermic. An endothermic reaction absorbs heat from its surroundings and has a positive ΔH. ***Entropy*** is a measure of energy that is unavailable for useful, chemical work. The entropy of an individual species is always positive and generally is larger for gases than for solids, and for more complex molecules than for simpler molecules. Reactions that produce a large number of simple, gaseous products usually have a positive ΔS.

The sign of ΔG indicates the direction in which a reaction moves to reach its equilibrium position. A reaction is thermodynamically favorable when its enthalpy, ΔH, decreases and its entropy, ΔS, increases. Substituting the inequalities $\Delta H < 0$ and $\Delta S > 0$ into Equation 6.2.2 shows that a reaction is thermodynamically favorable when ΔG is negative. When ΔG is positive the reaction is unfavorable as written (although the reverse reaction is favorable). A reaction at equilibrium has a ΔG of zero.

Equation 6.2.2 shows that the sign of ΔG depends on the signs of ΔH and of ΔS, and the temperature, T. The following table summarizes the possibilities.

ΔH	ΔS	ΔG
–	+	$\Delta G < 0$ at all temperatures
–	–	$\Delta G < 0$ at low temperatures only
+	+	$\Delta G < 0$ at high temperatures only
+	–	$\Delta G > 0$ at all temperatures

Note that the what constitutes "low temperatures" or "high temperatures" depends on the reaction.

As a reaction moves from its initial, non-equilibrium condition to its equilibrium position, its value of ΔG approaches zero. At the same time, the chemical species in the reaction experience a change in their concentrations. The Gibb's free energy, therefore, must be a function of the concentrations of reactants and products.

As shown in Equation 6.2.3, we can divide the Gibb's free energy, ΔG, into two terms.

$$\triangle G = \Delta G^\circ + RT \ln Q_r \tag{6.2.3}$$

The first term, ΔG°, is the change in the Gibb's free energy when each species in the reaction is in its ***standard state***, which we define as follows: gases with unit partial pressures, solutes with unit concentrations, and pure solids and pure liquids. The second term includes the reaction quotient, Q_r, which accounts for non-standard state pressures and concentrations. For reaction 6.2.1 the reaction quotient is

$$Q_r = \frac{[\mathrm{C}]^c[\mathrm{D}]^d}{[\mathrm{A}]^a[\mathrm{B}]^b} \tag{6.2.4}$$

where the terms in brackets are the concentrations of the reactants and products. Note that we define the reaction quotient with the products in the numerator and the reactants in the denominator. In addition, we raise the concentration of each species to a power equivalent to its stoichiometry in the balanced chemical reaction. For a gas, we use partial pressure in place of concentration. Pure solids and pure liquids do not appear in the reaction quotient.

Although not shown here, each concentration term in Equation 6.2.4 is divided by the corresponding standard state concentration; thus, the term $[\mathrm{C}]^c$ really means

$$\left\{\frac{[\mathrm{C}]}{[\mathrm{C}]^\circ}\right\}$$

where $[\mathrm{C}]^\circ$ is the standard state concentration for C. There are two important consequences of this: (1) the value of Q is unitless; and (2) the ratio has a value of 1 for a pure solid or a pure liquid. This is the reason that pure solids and pure liquids do not appear in the reaction quotient.

At equilibrium the Gibb's free energy is zero, and Equation 6.2.3 simplifies to

$$\triangle G^\circ = -RT \ln K$$

where K is an ***equilibrium constant*** that defines the reaction's equilibrium position. The equilibrium constant is just the reaction quotient's numerical value when we substitute equilibrium concentrations into Equation 6.2.4.

$$K = \frac{[\mathrm{C}]^c_{\mathrm{eq}}[\mathrm{D}]^d_{\mathrm{eq}}}{[\mathrm{A}]^a_{\mathrm{eq}}[\mathrm{B}]^b_{\mathrm{eq}}} \tag{6.2.5}$$

Here we include the subscript "eq" to indicate a concentration at equilibrium. Although generally we will omit the "eq" when we write an equilibrium constant expressions, it is important to remember that the value of K is determined by equilibrium concentrations.

As written, Equation 6.2.5 is a limiting law that applies only to infinitely dilute solutions where the chemical behavior of one species is unaffected by the presence of other species. Strictly speaking, Equation 6.2.5 is written in terms of activities instead of concentrations. We will return to this point in Chapter 6.9. For now, we will stick with concentrations as this convention already is familiar to you.

6.3: Manipulating Equilibrium Constants

We will take advantage of two useful relationships when we work with equilibrium constants. First, if we reverse a reaction's direction, the equilibrium constant for the new reaction is the inverse of that for the original reaction. For example, the equilibrium constant for the reaction

$$\mathrm{A} + 2\mathrm{B} \rightleftharpoons \mathrm{AB_2} \qquad K_1 = \frac{[\mathrm{AB_2}]}{[\mathrm{A}][\mathrm{B}]^2}$$

is the inverse of that for the reaction

$$\mathrm{AB_2} \rightleftharpoons \mathrm{A} + 2\mathrm{B} \qquad K_2 = (K_1)^{-1} = \frac{[\mathrm{A}][\mathrm{B}]^2}{[\mathrm{AB_2}]}$$

Second, if we add together two reactions to form a new reaction, the equilibrium constant for the new reaction is the product of the equilibrium constants for the original reactions.

$$A + C \rightleftharpoons AC \qquad K_3 = \frac{[AC]}{[A][C]}$$

$$\mathrm{AC} + \mathrm{C} \rightleftharpoons \mathrm{AC_2} \qquad K_4 = \frac{[\mathrm{AC_2}]}{[\mathrm{AC}][\mathrm{C}]}$$

$$\mathrm{A} + 2\mathrm{C} \rightleftharpoons \mathrm{AC_2} \qquad K_5 = K_3 \times K_4 = \frac{[\mathrm{AC}]}{[\mathrm{A}][\mathrm{C}]} \times \frac{[\mathrm{AC_2}]}{[\mathrm{AC}][\mathrm{C}]} = \frac{[\mathrm{AC_2}]}{[\mathrm{A}][\mathrm{C}]^2}$$

✔ Example 6.3.1

Calculate the equilibrium constant for the reaction

$$2\text{A} + \text{B} \rightleftharpoons \text{C} + 3\text{D}$$

given the following information

$$\begin{aligned} &\text{Rxn 1}: A + B \rightleftharpoons D && K_1 = 0.40 \\ &\text{Rxn 2}: A + E \rightleftharpoons C + D + F && K_2 = 0.10 \\ &\text{Rxn 3}: C + E \rightleftharpoons B && K_3 = 2.0 \\ &\text{Rxn 4}: F + C \rightleftharpoons D + B && K_4 = 5.0 \end{aligned}$$

Solution

The overall reaction is equivalent to

$$\text{Rxn 1} + \text{Rxn 2} - \text{Rxn 3} + \text{Rxn 4}$$

Subtracting a reaction is equivalent to adding the reverse reaction; thus, the overall equilibrium constant is

$$K = \frac{K_1 \times K_2 \times K_4}{K_3} = \frac{0.40 \times 0.10 \times 5.0}{2.0} = 0.10$$

? Exercise 6.3.1

Calculate the equilibrium constant for the reaction

$$C + D + F \rightleftharpoons 2A + 3B$$

using the equilibrium constants from Example 6.3.1 .

Answer

The overall reaction is equivalent to

$$\text{Rxn 4} - 2 \times \text{Rxn 1}$$

Subtracting a reaction is equivalent to adding the reverse reaction; thus, the overall equilibrium constant is

$$K = \frac{K_4}{(K_1)^2} = \frac{(5.0)}{(0.40)^2} = 31.25 \approx 31$$

6.4: Equilibrium Constants for Chemical Reactions

Several types of chemical reactions are important in analytical chemistry, either in preparing a sample for analysis or during the analysis. The most significant of these are precipitation reactions, acid–base reactions, complexation reactions, and oxidation–reduction reactions. In this section we review these reactions and their equilibrium constant expressions.

> Another common name for an oxidation–reduction reaction is a redox reaction, where "red" is short for reduction and "ox" is short for oxidation.

Precipitation Reactions

In a precipitation reaction, two or more soluble species combine to form an insoluble ***precipitate***. The most common precipitation reaction is a metathesis reaction in which two soluble ionic compounds exchange parts. For example, if we add a solution of lead nitrate, $Pb(NO_3)_2$, to a solution of potassium chloride, KCl, a precipitate of lead chloride, $PbCl_2$, forms. We usually write a precipitation reaction as a net ionic equation, which shows only the precipitate and those ions that form the precipitate; thus, the precipitation reaction for $PbCl_2$ is

$$Pb^{2+}(aq) + 2Cl^-(aq) \rightleftharpoons PbCl_2(s)$$

When we write the equilibrium constant for a precipitation reaction, we focus on the precipitate's solubility; thus, for $PbCl_2$, the solubility reaction is

$$PbCl_2(s) \rightleftharpoons Pb^{2+}(aq) + 2Cl^-(aq)$$

and its equilibrium constant, or ***solubility product***, K_{sp}, is

$$K_{sp} = [Pb^{2+}]\,[Cl^-]^2 \tag{6.4.1}$$

Even though it does not appear in the K_{sp} expression, it is important to remember that Equation 6.4.1 is valid only if $PbCl_2(s)$ is present and in equilibrium with Pb^{2+} and Cl^-. You will find values for selected solubility products in Appendix 10.

Acid–Base Reactions

A useful definition of acids and bases is that independently introduced in 1923 by Johannes Brønsted and Thomas Lowry. In the Brønsted-Lowry definition, an ***acid*** is a proton donor and a ***base*** is a proton acceptor. Note the connection between these definitions—defining a base as a proton acceptor implies there is an acid available to donate the proton. For example, in reaction 6.4.2 acetic acid, CH_3COOH, donates a proton to ammonia, NH_3, which serves as the base.

$$CH_3COOH(aq) + NH_3(aq) \rightleftharpoons NH_4^+(aq) + CH_3COO^-(aq) \tag{6.4.2}$$

When an acid and a base react, the products are a new acid and a new base. For example, the acetate ion, CH_3COO^-, in reaction 6.4.2 is a base that can accept a proton from the acidic ammonium ion, NH_4^+, forming acetic acid and ammonia. We call the acetate ion the conjugate base of acetic acid, and we call the ammonium ion the conjugate acid of ammonia.

Strong and Weak Acids

The reaction of an acid with its solvent (typically water) is an acid dissociation reaction. We divide acids into two categories—strong and weak—based on their ability to donate a proton to the solvent. A strong acid, such as HCl, almost completely transfers its proton to the solvent, which acts as the base.

$$HCl(aq) + H_2O(l) \rightarrow H_3O^+(aq) + Cl^-(aq)$$

We use a single arrow ($\rightarrow$) in place of the equilibrium arrow ($\rightleftharpoons$) because we treat HCl as if it dissociates completely in an aqueous solution. In water, the common strong acids are hydrochloric acid (HCl), hydroiodic acid (HI), hydrobromic acid (HBr), nitric acid (HNO_3), perchloric acid ($HClO_4$), and the first proton of sulfuric acid (H_2SO_4).

> The strength of an acid is a function of the acid and the solvent. For example, HCl does not act as a strong acid in methanol. In this case we use the equilibrium arrow when writing the acid–base reaction.
>
> $$HCl + CH_3OH \rightleftharpoons CH_3OH_2^+ + Cl^-$$

A weak acid, of which aqueous acetic acid is one example, does not completely donate its acidic proton to the solvent. Instead, most of the acid remains undissociated with only a small fraction present as the conjugate base.

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq)$$

The equilibrium constant for this reaction is an ***acid dissociation constant***, K_a, which we write as

$$K_a = \frac{[CH_3COO^-]\,[H_3O^+]}{[CH_3COOH]} = 1.75 \times 10^{-5}$$

The magnitude of K provides information about a weak acid's relative strength, with a smaller K_a corresponding to a weaker acid. The ammonium ion, NH_4^+, for example, has a K_a of 5.702×10^{-10} and is a weaker acid than acetic acid.

> Earlier we noted that we omit pure solids and pure liquids from equilibrium constant expressions. Because the solvent, H_2O, is not pure, you might wonder why we have not included it in acetic acid's K_a expression. Recall that we divide each term in an equilibrium constant expression by its standard state value. Because the concentration of H_2O is so large—it is approximately 55.5 mol/L—its concentration as a pure liquid and as a solvent are virtually identical. The ratio
>
> $$\frac{[H_2O]}{[H_2O]^\circ}$$
>
> is essentially 1.00.

A ***monoprotic*** weak acid, such as acetic acid, has only a single acidic proton and a single acid dissociation constant. Other acids, such as phosphoric acid, have multiple acidic protons, each characterized by an acid dissociation constant. We call such acids ***polyprotic***. Phosphoric acid, for example, has three acid dissociation reactions and three acid dissociation constants.

$$H_3PO_4(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + H_2PO_4^-(aq)$$

$$K_{a1} = \frac{[H_2PO_4^-]\,[H_3O^+]}{[H_3PO_4]} = 7.11 \times 10^{-3}$$

$$H_2PO_4^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HPO_4^{2-}(aq)$$

$$K_{a2} = \frac{[HPO_4^{2-}]\,[H_3O^+]}{[H_2PO_4^-]} = 6.32 \times 10^{-8}$$

$$HPO_4^{2-}(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + PO_4^{3-}(aq)$$

$$K_{a3} = \frac{[PO_4^{3-}]\,[H_3O^+]}{[HPO_4^{2-}]} = 4.5 \times 10^{-13}$$

The decrease in the acid dissociation constants from K_{a1} to K_{a3} tells us that each successive proton is harder to remove. Consequently, H_3PO_4 is a stronger acid than $H_2PO_4^-$, and $H_2PO_4^-$ is a stronger acid than HPO_4^{2-}.

Strong and Weak Bases

The most common example of a strong base is an alkali metal hydroxide, such as sodium hydroxide, NaOH, which completely dissociates to produce hydroxide ion.

$$NaOH(s) \rightarrow Na^+(aq) + OH^-(aq)$$

A weak base, such as the acetate ion, CH_3COO^-, only partially accepts a proton from the solvent, and is characterized by a ***base dissociation constant***, K_b. For example, the base dissociation reaction and the base dissociation constant for the acetate ion are

$$CH_3COO^-(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + CH_3COOH(aq)$$

$$K_b = \frac{[CH_3COOH]\,[OH^-]}{[CH_3COO^-]} = 5.71 \times 10^{-10}$$

A polyprotic weak base, like a polyprotic acid, has more than one base dissociation reaction and more than one base dissociation constant.

Amphiprotic Species

Some species can behave as either a weak acid or as a weak base. For example, the following two reactions show the chemical reactivity of the bicarbonate ion, HCO_3^-, in water.

$$HCO_3^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CO_3^{2-}(aq) \tag{6.4.3}$$

$$HCO_3^-(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + H_2CO_3(aq) \tag{6.4.4}$$

A species that is both a proton donor and a proton acceptor is called ***amphiprotic***. Whether an amphiprotic species behaves as an acid or as a base depends on the equilibrium constants for the competing reactions. For bicarbonate, the acid dissociation constant for reaction 6.4.3

$$K_{a2} = \frac{[CO_3^{2-}]\,[H_3O^+]}{[HCO_3^-]} = 4.69 \times 10^{-11}$$

is smaller than the base dissociation constant for reaction 6.4.4.

$$K_{b2} = \frac{[H_2CO_3]\,[OH^-]}{[HCO_3^-]} = 2.25 \times 10^{-8}$$

Because bicarbonate is a stronger base than it is an acid, we expect that an aqueous solution of HCO_3^- is basic.

Dissociation of Water

Water is an amphiprotic solvent because it can serve as an acid or as a base. An interesting feature of an amphiprotic solvent is that it is capable of reacting with itself in an acid–base reaction.

$$2H_2O(l) \rightleftharpoons H_3O^+(aq) + OH^-(aq) \tag{6.4.5}$$

We identify the equilibrium constant for this reaction as water's dissociation constant, K_w,

$$K_w = [H_3O^+]\,[OH^-] = 1.00 \times 10^{-14} \tag{6.4.6}$$

at a temperature of 24°C. The value of K_w varies substantially with temperature. For example, at 20°C K_w is 6.809×10^{-15}, while at 30°C K_w is 1.469×10^{-14}. At 25°C, K_w is 1.008×10^{-14}, which is sufficiently close to 1.00×10^{-14} that we can use the latter value with negligible error.

An important consequence of Equation 6.4.6 is that the concentration of H_3O^+ and the concentration of OH^- are related. If we know $[H_3O^+]$ for a solution, then we can calculate $[OH^-]$ using Equation 6.4.6.

Example 6.4.1

What is the $[OH^-]$ if the $[H_3O^+]$ is 6.12×10^{-5} M?

Solution

$$[OH^-] = \frac{K_w}{[H_3O^+]} = \frac{1.00 \times 10^{-14}}{6.12 \times 10^{-5}} = 1.63 \times 10^{-10}$$

The pH Scale

Equation 6.4.6 allows us to develop a ***pH scale*** ($pH = -\log[H_3O^+]$) that indicates a solution's acidity. When the concentrations of H_3O^+ and OH^- are equal a solution is neither acidic nor basic; that is, the solution is neutral. Letting

$$[H_3O^+] = [OH^-]$$

substituting into Equation 6.4.6

$$K_w = [H_3O^+]^2 = 1.00 \times 10^{-14}$$

and solving for $[H_3O^+]$ gives

$$[H_3O^+] = \sqrt{1.00 \times 10^{-14}} = 1.00 \times 10^{-7}$$

A neutral solution of water at 25°C has a hydronium ion concentration of 1.00×10^{-7} M and a pH of 7.00. In an acidic solution the concentration of H_3O^+ is greater than that for OH^-, which means that

$$[H_3O^+] > 1.00 \times 10^{-7}\text{M}$$

The pH of an acidic solution, therefore, is less than 7.00. A basic solution, on the other hand, has a pH greater than 7.00. Figure 6.4.1 shows the pH scale and pH values for some representative solutions.

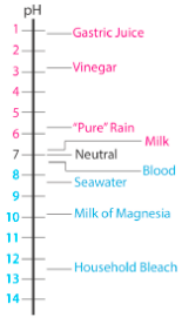


Figure 6.4.1 . Scale showing the pH value for representative solutions. Milk of Magnesia is a saturated solution of $Mg(OH)_2$.

Tabulating Values for K_a and K_b

A useful observation about weak acids and weak bases is that the strength of a weak base is inversely proportional to the strength of its conjugate weak acid. Consider, for example, the dissociation reactions of acetic acid and acetate.

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq) \tag{6.4.7}$$

$$CH_3COO^-(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + CH_3COOH(aq) \tag{6.4.8}$$

Adding together these two reactions gives the reaction

$$2H_2O(l) \rightleftharpoons H_3O^+(aq) + OH^-(aq)$$

for which the equilibrium constant is K_w. Because adding together two reactions is equivalent to multiplying their respective equilibrium constants, we may express K_w as the product of K_a for CH3COOH and K_b for CH_3COO^-.

$$K_w = K_{a,CH_3COOH} \times K_{b,CH_3COO^-}$$

For any weak acid, HA, and its conjugate weak base, A^-, we can generalize this to the following equation

$$K_w = K_{a,HA} \times K_{b,A^-} \tag{6.4.9}$$

where HA and A^- are a conjugate acid–base pair. The relationship between K_a and K_b for a conjugate acid–base pair simplifies our tabulation of acid and base dissociation constants. Appendix 11 includes acid dissociation constants for a variety of weak acids. To find the value of K_b for a weak base, use Equation 6.4.9 and the K_a value for its corresponding weak acid.

> A common mistake when using Equation 6.4.9 is to forget that it applies to a conjugate acid–base pair only.

✔ Example 6.4.2

Using Appendix 11, calculate values for the following equilibrium constants.

a. K_b for pyridine, C_5H_5N
b. K_b for dihydrogen phosphate, $H_2PO_4^-$

Solution

$$\text{(a) } K_{b,C_5H_5N} = \frac{K_w}{K_{a,C_5H_5NH^+}} = \frac{1.00 \times 10^{-14}}{5.90 \times 10^{-6}} = 1.69 \times 10^{-9}$$

$$\text{(b) } K_{b,H_2PO_4^-} = \frac{K_w}{K_{a,H_3PO_4}} = \frac{1.00 \times 10^{-14}}{7.11 \times 10^{-3}} = 1.41 \times 10^{-12}$$

> When finding the K_b value for a polyprotic weak base, be careful to choose the correct K_a value. Remember that Equation 6.4.9 applies to a conjugate acid–base pair only. The conjugate acid of $H_2PO_4^-$ is H_3PO_4, not HPO_4^{2-}.

? Exercise 6.4.1

Using Appendix 11, calculate K_b values for hydrogen oxalate, $HC_2O_4^-$, and for oxalate, $C_2O_4^{2-}$.

Answer

The K_b for hydrogen oxalate is

$$K_{b,HC_2O_4^-} = \frac{K_w}{K_{a,H_2C_2O_4}} = \frac{1.00 \times 10^{-14}}{5.60 \times 10^{-2}} = 1.79 \times 10^{-13}$$

and the K_b for oxalate is

$$K_{b,C_2O_4^{2-}} = \frac{K_w}{K_{a,HC_2O_4^-}} = \frac{1.00 \times 10^{-14}}{5.42 \times 10^{-5}} = 1.85 \times 10^{-10}$$

As we expect, the K_b value for $C_2O_4^{2-}$ is larger than that for $HC_2O_4^-$.

Complexation Reactions

A more general definition of acids and bases was proposed in 1923 by G. N. Lewis. The Brønsted-Lowry definition of acids and bases focuses on an acid's proton-donating ability and a base's proton-accepting ability. Lewis theory, on the other hand, uses the breaking and the forming of covalent bonds to describe acids and bases. In this treatment, an acid is an electron pair acceptor and a base in an electron pair donor. Although we can apply Lewis theory to the treatment of acid–base reactions, it is more useful for treating complexation reactions between metal ions and ligands.

The following reaction between the metal ion Cd^{2+} and the ***ligand*** NH_3 is typical of a complexation reaction.

$$Cd^{2+}(aq) + 4:NH_3(aq) \rightleftharpoons Cd(:NH_3)_4^{2+}(aq) \tag{6.4.10}$$

The product of this reaction is a ***metal–ligand complex***. In writing this reaction we show ammonia as $:NH_3$, using a pair of dots to emphasize the pair of electrons that it donates to Cd^{2+}. In subsequent reactions we will omit this notation.

Metal-Ligand Formation Constants

We characterize the formation of a metal–ligand complex by a ***formation constant***, K_f. For example, the complexation reaction between Cd^{2+} and NH_3, reaction 6.4.10, has the following equilibrium constant.

$$K_f = \frac{\left[Cd(NH_3)_4^{2+}\right]}{\left[Cd^{2+}\right]\left[NH_3\right]^4} = 5.5 \times 10^7 \tag{6.4.11}$$

The reverse of reaction 6.4.10 is a dissociation reaction, which we characterize by a ***dissociation constant***, K_d, that is the reciprocal of K_f.

Many complexation reactions occur in a stepwise fashion. For example, the reaction between Cd^{2+} and NH_3 involves four successive reactions.

$$Cd^{2+}(aq) + NH_3(aq) \rightleftharpoons Cd(NH_3)^{2+}(aq) \tag{6.4.12}$$

$$Cd(NH_3)^{2+}(aq) + NH_3(aq) \rightleftharpoons Cd(NH_3)_2^{2+}(aq) \tag{6.4.13}$$

$$Cd(NH_3)_2^{2+}(aq) + NH_3(aq) \rightleftharpoons Cd(NH_3)_3^{2+}(aq) \tag{6.4.14}$$

$$Cd(NH_3)_3^{2+}(aq) + NH_3(aq) \rightleftharpoons Cd(NH_3)_4^{2+}(aq) \tag{6.4.15}$$

To avoid ambiguity, we divide formation constants into two categories. A ***stepwise formation constant***, which we designate as K_i for the i^{th} step, describes the successive addition of one ligand to the metal–ligand complex from the previous step. Thus, the equilibrium constants for reactions 6.4.12–6.4.15 are, respectively, K_1, K_2, K_3, and K_4. An overall, or ***cumulative formation constant***, which we designate as β_i, describes the addition of i ligands to the free metal ion. The equilibrium constant in Equation 6.4.11 is correctly identified as β_4, where

$$\beta_4 = K_1 \times K_2 \times K_3 \times K_4$$

In general

$$\beta_n = K_1 \times K_2 \times \cdots \times K_n = \prod_{i=1}^{n} K_i$$

Stepwise and overall formation constants for selected metal–ligand complexes are in Appendix 12.

Metal-Ligand Complexation and Solubility

A formation constant describes the addition of one or more ligands to a free metal ion. To find the equilibrium constant for a complexation reaction that includes a solid, we combine appropriate K_{sp} and K_f expressions. For example, the solubility of AgCl increases in the presence of excess chloride ions as the result of the following complexation reaction.

$$\mathrm{AgCl}(s) + \mathrm{Cl}^-(aq) \rightleftharpoons \mathrm{Ag(Cl)}_2^-(aq) \tag{6.4.16}$$

We can write this reaction as the sum of three other equilibrium reactions with known equilibrium constants—the solubility of AgCl, which is described by its K_{sp} reaction

$$\mathrm{AgCl}(s) \rightleftharpoons \mathrm{Ag}^+(aq) + \mathrm{Cl}^-(aq)$$

and the stepwise formation of AgCl_2^-, which is described by $K_1$and K_2 reactions.

$$\mathrm{Ag}^+(aq) + \mathrm{Cl}^-(aq) \rightleftharpoons \mathrm{AgCl}(aq)$$

$$\mathrm{AgCl}(aq) + \mathrm{Cl}^-(aq) \rightleftharpoons \mathrm{AgCl}_2^-(aq)$$

The equilibrium constant for reaction 6.4.16, therefore, is $K_{sp} \times K_1 \times K_2$.

Example 6.4.3

Determine the value of the equilibrium constant for the reaction

$$\mathrm{PbCl_2}(s) \rightleftharpoons \mathrm{PbCl_2}(aq)$$

Solution

We can write this reaction as the sum of three other reactions. The first of these reactions is the solubility of $PbCl_2(s)$, which is described by its K_{sp} reaction.

$$\mathrm{PbCl_2}(s) \rightleftharpoons \mathrm{Pb}^{2+}(aq) + 2\mathrm{Cl}^-(aq)$$

The remaining two reactions are the stepwise formation of $PbCl_2(aq)$, which are described by K_1 and K_2.

$$\mathrm{Pb}^{2+}(aq) + \mathrm{Cl}^-(aq) \rightleftharpoons \mathrm{PbCl}^+(aq)$$

$$\mathrm{PbCl}^+(aq) + \mathrm{Cl}^-(aq) \rightleftharpoons \mathrm{PbCl_2}(aq)$$

Using values for K_{sp}, K_1, and K_2 from Appendix 10 and Appendix 12, we find that the equilibrium constant is

$$K = K_{sp} \times K_1 \times K_2 = \left(1.7 \times 10^{-5}\right) \times 38.9 \times 1.62 = 1.1 \times 10^{-3}$$

Exercise 6.4.2

What is the equilibrium constant for the following reaction? You will find appropriate equilibrium constants in Appendix 10 and Appendix 12.

$$\mathrm{AgBr}(s) + 2\mathrm{S_2O_3^{2-}}(aq) \rightleftharpoons \mathrm{Ag(S_2O_3)_2^{3-}}(aq) + \mathrm{Br}^-(aq)$$

Answer

We can write the reaction as a sum of three other reactions. The first reaction is the solubility of AgBr(s), which we characterize by its K_{sp}.

$$\mathrm{AgBr}(s) \rightleftharpoons \mathrm{Ag}^+(aq) + \mathrm{Br}^-(aq)$$

The remaining two reactions are the stepwise formation of $\mathrm{Ag(S_2O_3)_2^{3-}}$, which we characterize by K_1 and K_2.

$$\mathrm{Ag}^+(aq) + \mathrm{S_2O_3^{2-}}(aq) \rightleftharpoons \mathrm{Ag(S_2O_3)^-}(aq)$$

$$\mathrm{Ag(S_2O_3)^-}(aq) + \mathrm{S_2O_3^{2-}}(aq) \rightleftharpoons \mathrm{Ag(S_2O_3)_2^{3-}}(aq)$$

Using values for K_{sp}, K_1, and K_2 from Appendix 10 and Appendix 12, we find that the equilibrium constant for our reaction is

$$K = K_{sp} \times K_1 \times K_2 = \left(5.0 \times 10^{-13}\right)\left(6.6 \times 10^{8}\right)\left(7.1 \times 10^{4}\right) = 23$$

Oxidation–Reduction (Redox) Reactions

An oxidation–reduction reaction occurs when electrons move from one reactant to another reactant. As a result of this transfer of electrons, the reactants undergo a change in oxidation state. Those reactant that increases its oxidation state undergoes ***oxidation***, and the reactant that decreases its oxidation state undergoes ***reduction***. For example, in the following redox reaction between Fe^{3+} and oxalic acid, $H_2C_2O_4$, iron is reduced because its oxidation state changes from +3 to +2.

$$\begin{aligned} &2\mathrm{Fe}^{3+}(aq) + \mathrm{H_2C_2O_4}(aq) + 2\mathrm{H_2O}(l) \rightleftharpoons \\ &2\mathrm{Fe}^{2+}(aq) + 2\mathrm{CO_2}(g) + 2\mathrm{H_3O}^+(aq) \end{aligned} \tag{6.4.17}$$

Oxalic acid, on the other hand, is oxidized because the oxidation state for carbon increases from +3 in $H_2C_2O_4$ to +4 in CO_2.

We can divide a redox reaction, such as reaction 6.4.17, into separate ***half-reactions*** that show the oxidation and the reduction processes.

$$\mathrm{H_2C_2O_4}(aq) + 2\mathrm{H_2O}(l) \rightleftharpoons 2\mathrm{CO_2}(g) + 2\mathrm{H_3O}^+(aq) + 2e^-$$

$$\mathrm{Fe}^{3+}(aq) + e^- \rightleftharpoons \mathrm{Fe}^{2+}(aq)$$

It is important to remember, however, that an oxidation reaction and a reduction reaction always occur as a pair. We formalize this relationship by identifying as a ***reducing agent*** the reactant that is oxidized, because it provides the electrons for the reduction half-reaction. Conversely, the reactant that is reduced is an ***oxidizing agent***. In reaction 6.4.17, Fe^{3+} is the oxidizing agent and $H_2C_2O_4$ is the reducing agent.

The products of a redox reaction also have redox properties. For example, the Fe^{2+} in reaction 6.4.17 is oxidized to Fe^{3+} when CO_2 is reduced to $H_2C_2O_4$. Borrowing some terminology from acid–base chemistry, Fe^{2+} is the conjugate reducing agent of the oxidizing agent Fe^{3+}, and CO_2 is the conjugate oxidizing agent of the reducing agent $H_2C_2O_4$.

Thermodynamics of Redox Reactions

Unlike precipitation reactions, acid–base reactions, and complexation reactions, we rarely express the equilibrium position of a redox reaction with an equilibrium constant. Because a redox reaction involves a transfer of electrons from a reducing agent to an oxidizing agent, it is convenient to consider the reaction's thermodynamics in terms of the electron.

For a reaction in which one mole of a reactant undergoes oxidation or reduction, the net transfer of charge, Q, in coulombs is

$$Q = nF$$

where n is the moles of electrons per mole of reactant, and F is Faraday's constant (96485 C/mol). The free energy, ΔG, to move this charge, Q, over a change in ***potential***, E, is

$$\triangle G = EQ$$

The change in free energy (in kJ/mole) for a redox reaction, therefore, is

$$\Delta G = -nFE \tag{6.4.18}$$

where ΔG has units of kJ/mol. The minus sign in Equation 6.4.18 is the result of a different convention for assigning a reaction's favorable direction. In thermodynamics, a reaction is favored when ΔG is negative, but an oxidation-reduction reaction is favored when E is positive. Substituting Equation 6.4.18 into equation 6.2.3

$$-nFE = -nFE^\circ + RT \ln Q_r$$

and dividing by $-nF$, leads to the well-known ***Nernst equation***

$$E = E^\circ - \frac{RT}{nF} \ln Q_r$$

where E° is the potential under standard-state conditions. Substituting appropriate values for R and F, assuming a temperature of 25 °C (298 K), and switching from *ln* to *log* gives the potential in volts as

$$E = E^\circ - \frac{0.05916}{n} \log Q_r \tag{6.4.19}$$

Standard Potentials

A redox reaction's ***standard potential***, E°, provides an alternative way of expressing its equilibrium constant and, therefore, its equilibrium position. Because a reaction at equilibrium has a ΔG of zero, the potential, E, also is zero at equilibrium. Substituting these values into Equation 6.4.19 and rearranging provides a relationship between E° and K

$$E^\circ = \frac{0.05916}{n} \log K \tag{6.4.20}$$

> A standard potential is the potential when all species are in their standard states. You may recall that we define standard state conditions as follows: all gases have unit partial pressures, all solutes have unit concentrations, and all solids and liquids are pure.

We generally do not tabulate standard potentials for redox reactions. Instead, we calculate E° using the standard potentials for the corresponding oxidation half-reaction and reduction half-reaction. By convention, standard potentials are provided for reduction half-reactions. The standard potential for a redox reaction, E°, is

$$E^\circ = E^\circ_{red} - E^\circ_{ox}$$

where E°_{red} and E°_{ox} are the standard reduction potentials for the reduction half-reaction and the oxidation half-reaction.

Because we cannot measure the potential for a single half-reaction, we arbitrarily assign a standard reduction potential of zero to a reference half-reaction

$$2\text{H}_3\text{O}^+(aq) + 2e^- \rightleftharpoons 2\text{H}_2\text{O}(l) + \text{H}_2(g)$$

and report all other reduction potentials relative to this reference. Appendix 13 contains a list of selected standard reduction potentials. The more positive the standard reduction potential, the more favorable the reduction reaction is under standard state conditions. For example, under standard state conditions the reduction of Cu^{2+} to Cu (E° = +0.3419 V) is more favorable than the reduction of Zn^{2+} to Zn (E° = −0.7618 V).

✔ Example 6.4.4

Calculate (a) the standard potential, (b) the equilibrium constant, and (c) the potential when $[Ag^+]$ = 0.020 M and $[Cd^{2+}]$ = 0.050 M, for the following reaction at 25°C.

$$\text{Cd}(s) + 2\text{Ag}^+(aq) \rightleftharpoons 2\text{Ag}(s) + \text{Cd}^{2+}(aq)$$

Solution

(a) In this reaction Cd is oxidized and Ag^+ is reduced. The standard cell potential, therefore, is

$$E^\circ = E^\circ_{\text{Ag}^+/\text{Ag}} - E^\circ_{\text{Cd}^{2+}/\text{Cd}} = 0.7996 - (-0.4030) = 1.2026 \text{ V}$$

(b) To calculate the equilibrium constant we substitute appropriate values into Equation 6.4.20.

$$E^\circ = 1.2026 \text{ V} = \frac{0.05916 \text{ V}}{2} \log K$$

Solving for K gives the equilibrium constant as

$$\log K = 40.6558$$
$$K = 4.527 \times 10^{40}$$

(c) To calculate the potential when $[Ag^+]$ is 0.020 M and $[Cd^{2+}]$ is 0.050M, we use the appropriate relationship for the reaction quotient, Q_r, in Equation 6.4.19.

$$E = E^\circ - \frac{0.05916 \text{ V}}{n} \log \frac{[\text{Cd}^{2+}]}{[\text{Ag}^+]^2}$$
$$E = 1.2026 \text{ V} - \frac{0.05916 \text{ V}}{2} \log \frac{0.050}{(0.020)^2} = 1.14 \text{ V}$$

? Exercise 6.4.3

For the following reaction at 25°C

$$5Fe^{2+}(aq) + MnO_4^-(aq) + 8H^+(aq) \rightleftharpoons 5Fe^{3+}(aq) + Mn^{2+}(aq) + 4H_2O(l)$$

calculate (a) the standard potential, (b) the equilibrium constant, and (c) the potential under these conditions: [Fe^{2+}] = 0.50 M, [Fe^{3+}] = 0.10 M, [MnO_4^-] = 0.025 M, [Mn^{2+}] = 0.015 M, and a pH of 7.00. See Appendix 13 for standard state reduction potentials.

Answer

The two half-reactions are the oxidation of Fe^{2+} and the reduction of MnO_4^-.

$$Fe^{2+}(aq) \rightleftharpoons Fe^{3+}(aq) + e^-$$

$$MnO_4^-(aq) + 8H^+(aq) + 5e^- \rightleftharpoons Mn^{2+}(aq) + 4H_2O(l)$$

From Appendix 13, the standard state reduction potentials for these half-reactions are

$$E^\circ_{Fe^{3+}/Fe^{2+}} = 0.771 \text{ V and } E^\circ_{MnO_4^-/Mn^{2+}} = 1.51 \text{ V}$$

(a) The standard state potential for the reaction is

$$E^\circ = E^\circ_{MnO_4^-/Mn^{2+}} - E^\circ_{Fe^{3+}/Fe^{2+}} = 1.51 \text{ V} - 0.771 \text{ V} = 0.74 \text{ V}$$

(b) To calculate the equilibrium constant we substitute appropriate values into Equation 6.4.20.

$$E^\circ = 0.74 \text{ V} = \frac{0.05916}{5} \log K$$

Solving for K gives its value as 3.5×10^{62}.

(c) To calculate the potential under these non-standard state conditions, we make appropriate substitutions into the Nernst equation.

$$E = E^\circ - \frac{RT}{nF} \ln \frac{[Mn^{2+}]\,[Fe^{3+}]^5}{[MnO_4^-]\,[Fe^{2+}]^5[H^+]^8}$$

$$E = 0.74 - \frac{0.05916}{5} \log \frac{(0.015)(0.10)^5}{(0.025)(0.50)^5(1 \times 10^{-7})^8} = 0.12 \text{ V}$$

When writing precipitation, acid–base, and metal–ligand complexation reactions, we represent acidity as H_3O^+. Redox reactions more commonly are written using H^+ instead of H_3O^+. For the reaction in Exercise 6.4.3 , we could replace H^+ with H_3O^+ and increase the stoichiometric coefficient for H_2O from 4 to 12.

6.5: Le Châtelier's Principle

At a temperature of 25°C, acetic acid's dissociation reaction

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq)$$

has an equilibrium constant of

$$K_a = \frac{[CH_3COO^-]\,[H_3O^+]}{[CH_3COOH]} = 1.75 \times 10^{-5} \tag{6.5.1}$$

Because Equation 6.5.1 has three variables—[CH_3COOH], [CH_3COO^-], and [H_3O^+]—it does not have a unique mathematical solution. Nevertheless, although two solutions of acetic acid may have different values for [CH_3COOH], [CH_3COO^-], and [H_3O^+], each solution has the same value of K_a.

If we add sodium acetate to a solution of acetic acid, the concentration of CH_3COO^- increases, which suggests there is an increase in the value of K_a; however, because K_a must remain constant, the concentration of all three species in Equation 6.5.1 must change to restore K_a to its original value. In this case, a partial reaction of CH_3COO^- and H_3O^+ decreases their concentrations, increases the concentration of CH_3COOH, and reestablishes the equilibrium.

The observation that a system at equilibrium responds to an external action by reequilibrating itself in a manner that diminishes that action, is formalized as ***Le Châtelier's principle***. One common action is to change the concentration of a reactant or product for a system at equilibrium. As noted above for a solution of acetic acid, if we add a product to a reaction at equilibrium the system responds by converting some of the products into reactants. Adding a reactant has the opposite effect, resulting in the conversion of reactants to products.

When we add sodium acetate to a solution of acetic acid, we directly apply the action to the system. It is also possible to apply a change concentration indirectly. Consider, for example, the solubility of AgCl.

$$AgCl(s) \rightleftharpoons Ag^+(aq) + Cl^-(aq) \tag{6.5.2}$$

The effect on the solubility of AgCl of adding $AgNO_3$ is obvious, but what is the effect if we add a ligand that forms a stable, soluble complex with Ag^+? Ammonia, for example, reacts with Ag^+ as shown here

$$Ag^+(aq) + 2NH_3(aq) \rightleftharpoons Ag(NH_3)_2^+(aq) \tag{6.5.3}$$

Adding ammonia decreases the concentration of Ag^+ as the $Ag(NH_3)_2^+$ complex forms. In turn, a decrease in the concentration of Ag^+ increases the solubility of AgCl as reaction 6.5.2 reestablishes its equilibrium position. Adding together reaction 6.5.2 and reaction 6.5.3 clarifies the effect of ammonia on the solubility of AgCl, by showing ammonia as a reactant.

$$AgCl(s) + 2NH_3(aq) \rightleftharpoons Ag(NH_3)_2^+(aq) + Cl^-(aq) \tag{6.5.4}$$

So what is the effect on the solubility of AgCl of adding $AgNO_3$? Adding $AgNO_3$ increases the concentration of Ag^+ in solution. To reestablish equilibrium, some of the Ag^+ and Cl^- react to form additional AgCl; thus, the solubility of AgCl decreases. The solubility product, K_{sp}, of course, remains unchanged.

✔ Example 6.5.1

What happens to the solubility of AgCl if we add HNO_3 to the equilibrium solution defined by reaction 6.5.4?

Solution

Nitric acid is a strong acid, which reacts with ammonia as shown here

$$HNO_3(aq) + NH_3(aq) \rightleftharpoons NH_4^+(aq) + NO_3^-(aq)$$

Adding nitric acid lowers the concentration of ammonia. Decreasing ammonia's concentration causes reaction 6.5.4 to move from products to reactants, decreasing the solubility of AgCl.

Increasing or decreasing the partial pressure of a gas is the same as increasing or decreasing its concentration. Because the concentration of a gas depends on its partial pressure, and not on the total pressure of the system, adding or removing an inert gas has no effect on a reaction's equilibrium position.

We can use the ideal gas law to deduce the relationship between pressure and concentration. Starting with $PV = nRT$, we solve for the molar concentration

$$M = \frac{n}{V} = \frac{P}{RT}$$

Of course, this assumes that the gas is behaving ideally, which usually is a reasonable assumption under normal laboratory conditions.

Most reactions involve reactants and products dispersed in a solvent. If we change the amount of solvent by diluting the solution, then the concentrations of all reactants and products must increase; conversely, if we allow the solvent to evaporate partially, then the concentration of the solutes must increase. The effect of simultaneously changing the concentrations of all reactants and products is not intuitively as obvious as when we change the concentration of a single reactant or product. As an example, let's consider how diluting a solution affects the equilibrium position for the formation of the aqueous silver-amine complex (reaction 6.5.3). The equilibrium constant for this reaction is

$$\beta_2 = \frac{[Ag(NH_3)_2^+]_{eq}}{[Ag^+]_{eq}[NH_3]_{eq}^2} \tag{6.5.5}$$

where we include the subscript "eq" for clarification. If we dilute a portion of this solution with an equal volume of water, each of the concentration terms in Equation 6.5.5 is cut in half. The reaction quotient, Q_r, becomes

$$Q_r = \frac{0.5[Ag(NH_3)_2^+]_{eq}}{0.5[Ag^+]_{eq}(0.5)^2[NH_3]_{eq}^2} = \frac{0.5}{(0.5)^3} \times \frac{[Ag(NH_3)_2^+]_{eq}}{[Ag^+]_{eq}[NH_3]_{eq}^2} = 4\beta_2 \tag{6.5.6}$$

Because Q_r is greater than β_2, equilibrium is reestablished by shifting the reaction to the left, decreasing the concentration of $Ag(NH_3)_2^+$. Note that the new equilibrium position lies toward the side of the equilibrium reaction that has the greatest number of solute particles (one Ag^+ ion and two molecules of NH_3 versus a single metal-ligand complex). If we concentrate the solution of $Ag(NH_3)_2^+$ by evaporating some of the solvent, equilibrium is reestablished in the opposite direction. This is a general conclusion that we can apply to any reaction. Increasing volume always favors the direction that produces the greatest number of particles, and decreasing volume always favors the direction that produces the fewest particles. If the number of particles is the same on both sides of the reaction, then the equilibrium position is unaffected by a change in volume.

6.6: Ladder Diagrams

When we develop or evaluate an analytical method, we often need to understand how the chemistry that takes place affects our results. Suppose we wish to isolate Ag^+ by precipitating it as AgCl. If we also need to control pH, then we must use a reagent that does not adversely affect the solubility of AgCl. It is a mistake to use NH_3 to adjust the pH, for example, because it increases the solubility of AgCl (see reaction 6.5.4). One of the primary sources of determinate errors in many analytical methods is failing to account for potential chemical interferences.

In this section we introduce the ladder diagram as a simple graphical tool for visualizing equilibrium chemistry. We will use ladder diagrams to determine what reactions occur when we combine several reagents, to estimate the approximate composition of a system at equilibrium, and to evaluate how a change to solution conditions might affect an analytical method.

Although not specifically on the topic of ladder diagrams as developed in this section, the following papers provide appropriate background information: (a) Runo, J. R.; Peters, D. G. *J. Chem. Educ.* **1993**, *70*, 708–713; (b) Vale, J.; Fernández-Pereira, C.; Alcalde, M. *J. Chem. Educ.* **1993**, *70*, 790–795; (c) Fernández-Pereira, C.; Vale, J. *Chem. Educator* **1996**, *6*, 1–18; (d) Fernández- Pereira, C.; Vale, J.; Alcalde, M. *Chem. Educator* **2003**, *8*, 15–21; (e) Fernández-Pereira, C.; Alcalde, M.; Villegas, R.; Vale, J. *J. Chem. Educ.* **2007**, *84*, 520–525. Ladder diagrams are a great tool for helping you to think intuitively about analytical chemistry. We will make frequent use of them in the chapters to follow.

Ladder Diagrams for Acid–Base Equilibria

Let's use acetic acid, CH_3COOH, to illustrate the process we will use to draw and to interpret an acid–base ladder diagram. Before we draw the diagram, however, let's consider the equilibrium reaction in more detail. Acetic acid's acid dissociation reaction and equilibrium constant expression are

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq)$$

$$K_a = \frac{[CH_3COO^-]\,[H_3O^+]}{[CH_3COOH]} = 1.75 \times 10^{-5}$$

First, let's take the logarithm of each term in this equation and multiply through by −1

$$-\log K_a = 4.76 = -\log[H_3O^+] - \log\frac{[CH_3COO^-]}{[CH_3COOH]}$$

Now, let's replace $-\log[H_3O^+]$ with pH and rearrange the equation to obtain the result shown here.

$$pH = 4.76 + \log\frac{[CH_3COO^-]}{[CH_3COOH]} \tag{6.6.1}$$

Equation 6.6.1 tells us a great deal about the relationship between pH and the relative amounts of acetic acid and acetate at equilibrium. If the concentrations of CH_3COOH and CH_3COO^- are equal, then Equation 6.6.1 reduces to

$$pH = 4.76 + \log(1) = 4.76 + 0 = 4.76$$

If the concentration of CH_3COO^- is greater than that of CH_3COOH, then the log term in Equation 6.6.1 is positive and the pH is greater than 4.76. This is a reasonable result because we expect the concentration of the conjugate base, CH_3COO^-, to increase as the pH increases. Similar reasoning will convince you that the pH is less than 4.76 when the concentration of CH_3COOH exceeds that of CH_3COO^-.

Now we are ready to construct acetic acid's ladder diagram (Figure 6.6.1). First, we draw a vertical arrow that represents the solution's pH, with smaller (more acidic) pH levels at the bottom and larger (more basic) pH levels at the top. Second, we draw a horizontal line at a pH equal to acetic acid's pK_a value. This line, or step on the ladder, divides the pH axis into regions where either CH_3COOH or CH_3COO^- is the predominate species. This completes the ladder diagram.

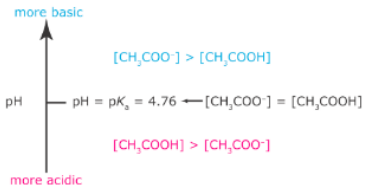


Figure 6.6.1 . Acid–base ladder diagram for acetic acid showing the relative concentrations of CH_3COOH and CH_3COO^-. A simpler version of this ladder diagram dispenses with the equalities and inequalities and shows only the predominate species in each region.

Using the ladder diagram, it is easy to identify the predominate form of acetic acid at any pH. At a pH of 3.5, for example, acetic acid exists primarily as CH_3COOH. If we add sufficient base to the solution such that the pH increases to 6.5, the predominate form of acetic acid is CH_3COO^-.

✔ Example 6.6.1

Draw a ladder diagram for the weak base *p*-nitrophenolate and identify its predominate form at a pH of 6.00.

Solution

To draw a ladder diagram for a weak base, we simply draw the ladder diagram for its conjugate weak acid. From Appendix 11, the pK_a for *p*-nitrophenol is 7.15. The resulting ladder diagram is shown in Figure 6.6.2 . At a pH of 6.00, *p*-nitrophenolate is present primarily in its weak acid form.

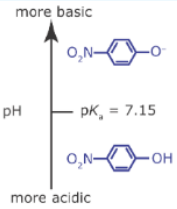


Figure 6.6.2 . Acid–base ladder diagram for *p*-nitrophenolate.

? Exercise 6.6.1

Draw a ladder diagram for carbonic acid, H_2CO_3. Because H_2CO_3 is a diprotic weak acid, your ladder diagram will have two steps. What is the predominate form of carbonic acid when the pH is 7.00? Relevant equilibrium constants are in Appendix 11.

Answer

A ladder diagram is particularly useful for evaluating the reactivity between a weak acid and a weak base. Figure 6.6.3 , for example, shows a single ladder diagram for acetic acid/acetate and for *p*-nitrophenol/*p*-nitrophenolate. An acid and a base can not co-exist if their respective areas of predominance do not overlap. If we mix together solutions of acetic acid and sodium *p*-nitrophenolate, the reaction

$$\mathrm{C_6H_4NO_2^-}(aq) + \mathrm{CH_3COOH}(aq) \rightleftharpoons \mathrm{CH_3COO^-}(aq) + \mathrm{C_6H_4NO_2H}(aq) \tag{6.6.2}$$

occurs because the areas of predominance for acetic acid and *p*-nitrophenolate do not overlap. The solution's final composition depends on which species is the limiting reagent. The following example shows how we can use the ladder diagram in Figure 6.6.3 to evaluate the result of mixing together solutions of acetic acid and *p*-nitrophenolate.

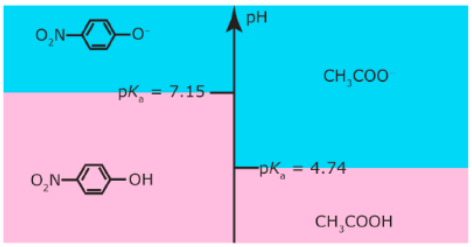


Figure 6.6.3 . Acid–base ladder diagram showing the areas of predominance for acetic acid/acetate and for *p*-nitrophenol/*p*-nitrophenolate. The areas shaded in blue shows the pH range where the weak bases are the predominate species; the weak acid forms are the predominate species in the areas shaded in pink.

✔ Example 6.6.2

Predict the approximate pH and the final composition after mixing together 0.090 moles of acetic acid and 0.040 moles of *p*-nitrophenolate.

Solution

The ladder diagram in Figure 6.6.3 indicates that the reaction between acetic acid and *p*-nitrophenolate is favorable. Because acetic acid is in excess, we assume the reaction of *p*-nitrophenolate to *p*-nitrophenol is complete. At equilibrium essentially no *p*-nitrophenolate remains and there are 0.040 mol of *p*-nitrophenol. Converting *p*-nitrophenolate to *p*-nitrophenol consumes 0.040 moles of acetic acid; thus

$$\text{moles } \mathrm{CH_3COOH} = 0.090 - 0.040 = 0.050 \text{ mol}$$
$$\text{moles } \mathrm{CH_3COO^-} = 0.040 \text{ mol}$$

According to the ladder diagram, the pH is 4.74 when there are equal amounts of CH_3COOH and CH_3COO^-. Because we have slightly more CH_3COOH than CH_3COO^-, the pH is slightly less than 4.74.

? Exercise 6.6.2

Using Figure 6.6.3 , predict the approximate pH and the composition of the solution formed by mixing together 0.090 moles of *p*-nitrophenolate and 0.040 moles of acetic acid.

Answer

The ladder diagram in Figure 6.6.3 indicates that the reaction between acetic acid and *p*-nitrophenolate is favorable. Because *p*-nitrophenolate is in excess, we assume the reaction of acetic acid to acetate is complete. At equilibrium essentially no acetic acid remains and there are 0.040 moles of acetate. Converting acetic acid to acetate consumes 0.040 moles of *p*-nitrophenolate; thus

$$\text{moles } p\text{-nitrophenolate } = 0.090 - 0.040 = 0.050 \text{ mol}$$

$$\text{moles } p\text{-nitrophenol } = 0.040 \text{ mol}$$

According to the ladder diagram for this system, the pH is 7.15 when there are equal concentrations of *p*-nitrophenol and *p*-nitrophenolate. Because we have slightly more *p*-nitrophenolate than we have *p*-nitrophenol, the pH is slightly greater than 7.15.

If the areas of predominance for an acid and a base overlap, then we do not expect that much of a reaction will occur. For example, if we mix together solutions of CH_3COO^- and *p*-nitrophenol, we do not expect a significant change in the moles of either reagent. Furthermore, the pH of the mixture must be between 4.76 and 7.15, with the exact pH depending upon the relative amounts of CH_3COO^- and *p*-nitrophenol.

We also can use an acid–base ladder diagram to evaluate the effect of pH on other equilibria. For example, the solubility of CaF_2

$$CaF_2(s) \rightleftharpoons Ca^{2+}(aq) + 2F^-(aq)$$

is affected by pH because F^- is a weak base. From Le Châtelier's principle, we know that converting F^- to HF will increase the solubility of CaF_2. To minimize the solubility of CaF_2 we need to maintain the solution's pH so that F^- is the predominate species. The ladder diagram for HF (Figure 6.6.4) shows us that maintaining a pH of more than 3.17 will minimize solubility losses.

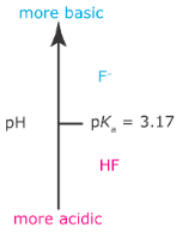


Figure 6.6.4 . Acid–base ladder diagram for HF. To minimize the solubility of CaF_2, we need to keep the pH above 3.17, with more basic pH levels leading to smaller solubility losses. See Chapter 8 for a more detailed discussion.

Ladder Diagrams for Complexation Equilibria

We can apply the same principles for constructing and interpreting an acid–base ladder diagram to equilibria that involve metal–ligand complexes. For a complexation reaction we define the ladder diagram's scale using the concentration of uncomplexed, or free ligand, pL. Using the formation of $Cd(NH_3)^{2+}$ as an example

$$Cd^{2+}(aq) + NH_3(aq) \rightleftharpoons Cd(NH_3)^{2+}(aq)$$

we can show that log K_1 is the dividing line between the areas of predominance for Cd^{2+} and for $Cd(NH_3)^{2+}$.

$$K_1 = 3.55 \times 10^2 = \frac{\left[Cd(NH_3)^{2+}\right]}{\left[Cd^{2+}\right]\left[NH_3\right]}$$

$$\log K_1 = \log\left(3.55 \times 10^2\right) = \log \frac{\left[Cd(NH_3)^{2+}\right]}{\left[Cd^{2+}\right]} - \log[NH_3]$$

$$\log K_1 = 2.55 = \log \frac{\left[Cd(NH_3)^{2+}\right]}{\left[Cd^{2+}\right]} + pNH_3$$

$$pNH_3 = \log K_1 + \log \frac{\left[Cd^{2+}\right]}{\left[Cd(NH_3)^{2+}\right]} = 2.55 + \log \frac{\left[Cd^{2+}\right]}{\left[Cd(NH_3)^{2+}\right]}$$

Thus, Cd^{2+} is the predominate species when pNH_3 is greater than 2.55 (a concentration of NH_3 smaller than 2.82×10^{-3} M) and for a pNH_3 value less than 2.55, $Cd(NH_3)^{2+}$ is the predominate species. Figure 6.6.5 shows a complete metal–ligand ladder diagram for Cd^{2+} and NH_3 that includes additional Cd–NH_3 complexes.

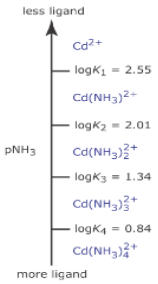


Figure 6.6.5 . Metal–ligand ladder diagram for Cd^{2+}–NH_3 complexation reactions. Note that higher-order complexes form when pNH_3 is smaller (which corresponds to larger concentrations of NH_3).

Example 6.6.3

Draw a single ladder diagram for the $Ca(EDTA)^{2-}$ and the $Mg(EDTA)^{2-}$ metal–ligand complexes. Use your ladder diagram to predict the result of adding 0.080 moles of Ca^{2+} to 0.060 moles of $Mg(EDTA)^{2-}$. EDTA is an abbreviation for the ligand ethylenediaminetetraacetic acid.

Solution

Figure 6.6.6 shows the ladder diagram for this system of metal–ligand complexes. Because the predominance regions for Ca^{2+} and $Mg(EDTA)^{2-}$ do not overlap, the reaction

$$Ca^{2+}(aq) + Mg(EDTA)^{2-}(aq) \rightleftharpoons Ca(EDTA)^{2-}(aq) + Mg^{2+}(aq)$$

proceeds essentially to completion. Because Ca^{2+} is the excess reagent, the composition of the final solution is approximately

$$\text{moles } Ca^{2+} = 0.080 - 0.060 = 0.020 \text{ mol}$$

$$\text{moles } Ca(EDTA)^{2-} = 0.060 \text{ mol}$$

$$\text{moles } Mg^{2+} = 0.060 \text{ mol}$$

$$\text{moles } Mg(EDTA)^{2-} = 0 \text{ mol}$$

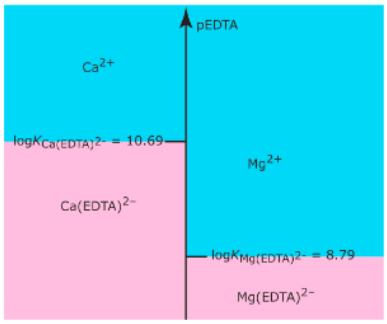


Figure 6.6.6 . Metal–ligand ladder diagram for $Ca(EDTA)^{2-}$ and for $Mg(EDTA)^{2-}$. The areas shaded in blue show the pEDTA range where the free metal ions are the predominate species; the metal–ligand complexes are the predominate species in the areas shaded in pink.

The metal–ligand ladder diagram in Figure 6.6.5 uses stepwise formation constants. We also can construct a ladder diagram using cumulative formation constants. For example, the first three stepwise formation constants for the reaction of Zn^{2+} with NH_3

$$Zn^{2+}(aq) + NH_3(aq) \rightleftharpoons Zn(NH_3)^{2+}(aq) \quad K_1 = 1.6 \times 10^2$$

$$Zn(NH_3)^{2+}(aq) + NH_3(aq) \rightleftharpoons Zn(NH_3)_2^{2+}(aq) \quad K_2 = 1.95 \times 10^2$$

$$Zn(NH_3)_2^{2+}(aq) + NH_3(aq) = Zn(NH_3)_3^{2+}(aq) \quad K_3 = 2.3 \times 10^2$$

suggests that the formation of $Zn(NH_3)_3^{2+}$ is more favorable than the formation of $Zn(NH_3)^{2+}$ or $Zn(NH_3)_2^{2+}$. For this reason, the equilibrium is best represented by the cumulative formation reaction shown here.

$$Zn^{2+}(aq) + 3NH_3(aq) \rightleftharpoons Zn(NH_3)_3^{2+}(aq) \quad \beta_3 = 7.2 \times 10^6$$

Because K_3 is greater than K_2, which is greater than K_1, the formation of the metal-ligand complex $Zn(NH_3)_3^{2+}$ is more favorable than the formation of the other metal ligand complexes. For this reason, at lower values of pNH_3 the concentration of $Zn(NH_3)_3^{2+}$ is larger than the concentrations of $Zn(NH_3)^{2+}$ or $Zn(NH_3)_2^{2+}$. The value of β_3 is

$$\beta_3 = K_1 \times K_2 \times K_3$$

To see how we incorporate this cumulative formation constant into a ladder diagram, we begin with the reaction's equilibrium constant expression.

$$\beta_3 = \frac{\left[Zn(NH_3)_3^{2+}\right]}{\left[Zn^{2+}\right]\left[NH_3\right]^3}$$

Taking the log of each side

$$\log \beta_3 = \log \frac{\left[Zn(NH_3)_3^{2+}\right]}{\left[Zn^{2+}\right]} - 3\log[NH_3]$$

and rearranging gives

$$pNH_3 = \frac{1}{3}\log \beta_3 + \frac{1}{3}\log \frac{\left[Zn^{2+}\right]}{\left[Zn(NH_3)_3^{2+}\right]}$$

When the concentrations of Zn and $Zn(NH_3)_3^{2+}$ are equal, then

$$pNH_3 = \frac{1}{3}\log \beta_3 = 2.29$$

In general, for the metal–ligand complex ML_n, the step for a cumulative formation constant is

$$\mathrm{pL} = \frac{1}{n}\log\beta_n$$

Figure 6.6.7 shows the complete ladder diagram for the Zn^{2+}–NH_3 system.

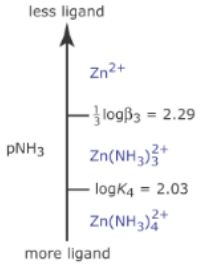


Figure 6.6.7 . Ladder diagram for Zn^{2+}–NH_3 metal–ligand complexation reactions showing both a step based on a cumulative formation constant and a step based on a stepwise formation constant.

Ladder Diagrams for Oxidation/Reduction Equilibria

We also can construct ladder diagrams to help us evaluate redox equilibria. Figure 6.6.8 shows a typical ladder diagram for two half-reactions in which the scale is the potential, *E*.

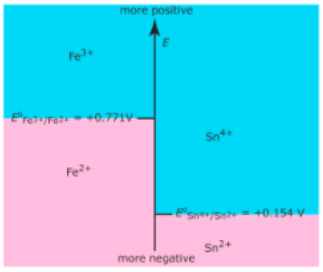


Figure 6.6.8 . Redox ladder diagram for Fe^{3+}/Fe^{2+} and for Sn^{4+}/Sn^{2+}. The areas shaded in blue show the potential range where the oxidized forms are the predominate species; the reduced forms are the predominate species in the areas shaded in pink. Note that a more positive potential favors the oxidized form.

The Nernst equation defines the areas of predominance. Using the Fe^{3+}/Fe^{2+} half-reaction as an example, we write

$$E = E^\circ - \frac{RT}{nF}\ln\frac{[\mathrm{Fe}^{2+}]}{[\mathrm{Fe}^{3+}]} = 0.771 - 0.05916\log\frac{[\mathrm{Fe}^{2+}]}{[\mathrm{Fe}^{3+}]}$$

At a potential more positive than the standard state potential, the predominate species is Fe^{3+}, whereas Fe^{2+} predominates at potentials more negative than E°. When coupled with the step for the Sn^{4+}/Sn^{2+} half-reaction we see that Sn^{2+} is a useful reducing agent for Fe^{3+}. If Sn^{2+} is in excess, the potential of the resulting solution is near +0.154 V.

Because the steps on a redox ladder diagram are standard state potentials, a complication arises if solutes other than the oxidizing agent and reducing agent are present at non-standard state concentrations. For example, the potential for the half-reaction

$$\mathrm{UO_2^{2+}}(aq) + 4\mathrm{H_3O^+}(aq) + 2e^- \rightleftharpoons \mathrm{U^{4+}}(aq) + 6\mathrm{H_2O}(l)$$

depends on the solution's pH. To define areas of predominance in this case we begin with the Nernst equation

$$E = +0.327 - \frac{0.05916}{2}\log\frac{[\mathrm{U^{4+}}]}{[\mathrm{UO_2^{2+}}]\,[\mathrm{H_3O^+}]^4}$$

and factor out the concentration of H_3O^+.

$$E = +0.327 + \frac{0.05916}{2}\log[\mathrm{H_3O^+}]^4 - \frac{0.05916}{2}\log\frac{[\mathrm{U^{4+}}]}{[\mathrm{UO_2^{2+}}]}$$

From this equation we see that the area of predominance for UO_2^{2+} and U^{4+} is defined by a step at a potential where $[U^{4+}] = [UO_2^{2+}]$.

$$E = +0.327 + \frac{0.05916}{2}\log[\mathrm{H_3O^+}]^4 = +0.327 - 0.1183\mathrm{pH}$$

Figure 6.6.9 shows how pH affects the step for the UO_2^{2+} /U^{4+} half-reaction.

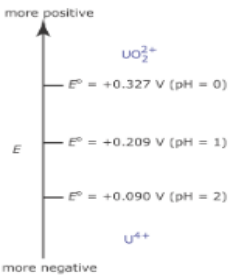


Figure 6.6.9 . Redox ladder diagram for the UO_2^{2+}/U^{4+} half-reaction showing the effect of pH on the step defined by the standard state's potential.

6.7: Solving Equilibrium Problems

Ladder diagrams are a useful tool for evaluating chemical reactivity and for providing a reasonable estimate of a chemical system's composition at equilibrium. If we need a more exact quantitative description of the equilibrium condition, then a ladder diagram is insufficient; instead, we need to find an algebraic solution. In this section we will learn how to set-up and solve equilibrium problems. We will start with a simple problem and work toward more complex problems.

A Simple Problem: The Solubility of $Pb(IO_3)_2$

If we place an insoluble compound such as $Pb(IO_3)_2$ in deionized water, the solid dissolves until the concentrations of Pb^{2+} and IO_3^- satisfy the solubility product for $Pb(IO_3)_2$. At equilibrium the solution is saturated with $Pb(IO_3)_2$, which means simply that no more solid can dissolve. How do we determine the equilibrium concentrations of Pb^{2+} and IO_3^-, and what is the molar solubility of $Pb(IO_3)_2$ in this saturated solution?

> When we first add solid $Pb(IO_3)_2$ to water, the concentrations of Pb^{2+} and IO_3^- are zero and the reaction quotient, Q_r, is
>
> $$Q_r = \left[Pb^{2+}\right]\left[IO_3^-\right]^2 = 0$$
>
> As the solid dissolves, the concentrations of these ions increase, but Q_r remains smaller than K_{sp}. We reach equilibrium and "satisfy the solubility product" when $Q_r = K_{sp}$.

We begin by writing the equilibrium reaction and the solubility product expression for $Pb(IO_3)_2$.

$$Pb(IO_3)_2(s) \rightleftharpoons Pb^{2+}(aq) + 2IO_3^-(aq)$$

e

$$K_{sp} = \left[Pb^{2+}\right]\left[IO_3^-\right]^2 = 2.5 \times 10^{-13} \tag{6.7.1}$$

As $Pb(IO_3)_2$ dissolves, two IO_3^- ions form for each ion of Pb^{2+}. If we assume that the change in the molar concentration of Pb^{2+} at equilibrium is x, then the change in the molar concentration of IO_3^- is $2x$. The following table helps us keep track of the initial concentrations, the change in con- centrations, and the equilibrium concentrations of Pb2+ and IO_3^-.

concentrations	$Pb(IO_3)_2$ (s)	⇌	Pb^{2+} (aq)	+	$2IO_3^-$ (aq)
initial	solid		0		0
change	solid		$+x$		$+2x$
equilibrium	solid		x		$2x$

> Because a solid, such as $Pb(IO_3)_2$, does not appear in the solubility product expression, we do not need to keep track of its concentration. Remember, however, that the K_{sp} value applies only if there is some solid $Pb(IO_3)_2$ present at equilibrium.

Substituting the equilibrium concentrations into Equation 6.7.1 and solving gives

$$(x)(2x)^2 = 4x^3 = 2.5 \times 10^{-13}$$

$$x = 3.97 \times 10^{-5}$$

Substituting this value of x back into the equilibrium concentration expressions for Pb^{2+} and IO_3^- gives their concentrations as

$$\left[Pb^{2+}\right] = x = 4.0 \times 10^{-5}\,\text{M and } \left[IO_3^-\right] = 2x = 7.9 \times 10^{-5}$$

Because one mole of $Pb(IO_3)_2$ contains one mole of Pb^{2+}, the molar solubility of $Pb(IO_3)_2$ is equal to the concentration of Pb^{2+}, or 4.0×10^{-5} M.

> We can express a compound's solubility in two ways: as its molar solubility (mol/L) or as its mass solubility (g/L). Be sure to express your answer clearly.

? Exercise 6.7.1

Calculate the molar solubility and the mass solubility for Hg_2Cl_2, given the following solubility reaction and K_{sp} value.

$$Hg_2Cl_2(s) \rightleftharpoons Hg_2^{2+}(aq) + 2Cl^-(aq) \quad K_{sp} = 1.2 \times 10^{-8}$$

Answer

A More Complex Problem: The Common Ion Effect

Calculating the solubility of $Pb(IO_3)_2$ in deionized water is a straightforward problem because the solid's dissolution is the only source of Pb^{2+} and IO_3^-. But what if we add $Pb(IO_3)_2$ to a solution of 0.10 M $Pb(NO_3)_2$? Before we set-up and solve this problem algebraically, think about the system's chemistry and decide whether the solubility of $Pb(IO_3)_2$ will increase, decrease, or remain the same. Beginning a problem by thinking about the likely answer is a good habit to develop. Knowing what answers are reasonable will help you spot errors in your calculations and give you more confidence that your solution to a problem is correct. Because the solution already contains a source of Pb^{2+}, we can use Le Châtelier's principle to predict that the solubility of $Pb(IO_3)_2$ is smaller than that in our previous problem.

We begin by setting up a table to help us keep track of the concentrations of Pb^{2+} and IO_3^- as this system moves toward and reaches equilibrium.

concentrations	$Pb(IO_3)_2$ (s)	⇌	Pb^{2+} (aq)	+	$2IO_3^-$ (aq)
initial	solid		0.10		0
change	solid		$+x$		$+2x$
equilibrium	solid		$0.10 + x$		$2x$

Substituting the equilibrium concentrations into Equation 6.7.1

$$(0.10 + x)(2x)^2 = 2.5 \times 10^{-13}$$

and multiplying out the terms on the equation's left side leaves us with

$$4x^3 + 0.40x^2 = 2.5 \times 10^{-13} \tag{6.7.2}$$

This is a more difficult equation to solve than that for the solubility of $Pb(IO_3)_2$ in deionized water, and its solution is not immediately obvious. We can find a rigorous solution to Equation 6.7.2 using computational software packages and spreadsheets, some of which are described in Chapter 6.10.

> There are several approaches to solving cubic equations, but none are computationally easy using just paper and pencil.

How might we solve Equation 6.7.2 if we do not have access to a computer? One approach is to use our understanding of chemistry to simplify the problem. From Le Châtelier's principle we know that a large initial concentration of Pb^{2+} will decrease significantly the solubility of $Pb(IO_3)_2$. One reasonable assumption is that the initial concentration of Pb^{2+} is very close to its equilibrium concentration. If this assumption is correct, then the following approximation is reasonable

$$\left[Pb^{2+}\right] = 0.10 + x \approx 0.10$$

Substituting this approximation into Equation 6.7.1 and solving for *x* gives

$$(0.10)(2x)^2 = 0.4x^2 = 2.5 \times 10^{-13}$$

$$x = 7.91 \times 10^{-7}$$

Before we accept this answer, we must verify that our approximation is reasonable. The difference between the actual concentration of Pb^{2+}, which is 0.10 + *x* M, and our assumption that the concentration of Pb^{2+} is 0.10 M is 7.9×10^{-7}, or 7.9×10^{-4} % of the assumed concentration. This is a negligible error. If we accept the result of our calculation, we find that the equilibrium concentrations of Pb^{2+} and IO_3^- are

$$\left[Pb^{2+}\right] = 0.10 + x \approx 0.10 \text{ M and } \left[IO_3^-\right] = 2x = 1.6 \times 10^{-6} \text{ M}$$

$$\begin{aligned} \%\text{ error } &= \frac{\text{actual } - \text{ assumed}}{\text{assumed}} \times 100 \\ &= \frac{(0.10 + x) - 0.10}{0.10} \times 100 \\ &= \frac{7.91 \times 10^{-7}}{0.10} \times 100 \\ &= 7.91 \times 10^{-4}\% \end{aligned}$$

The molar solubility of $Pb(IO_3)_2$ is equal to the additional concentration of Pb^{2+} in solution, or 7.9×10^{-4} mol/L. As expected, we find that $Pb(IO_3)_2$ is less soluble in the presence of a solution that already contains one of its ions. This is known as the ***common ion effect***.

As outlined in the following example, if an approximation leads to an error that is unacceptably large, then we can extend the process of making and evaluating approximations.

> One "rule of thumb" when making an approximation is that it should not introduce an error of more than ±5%. Although this is not an unreasonable choice, what matters is that the error makes sense within the context of the problem you are solving.

✔ Example 6.7.1

Calculate the solubility of $Pb(IO_3)_2$ in 1.0×10^{-4} M $Pb(NO_3)_2$.

Solution

If we let *x* equal the change in the concentration of Pb^{2+}, then the equilibrium concentrations of Pb^{2+} and IO_3^- are

$$\left[Pb^{2+}\right] = 1.0 \times 10^{-4} + x \text{ and } \left[IO_3^-\right] = 2x$$

Substituting these concentrations into Equation 6.7.1 leaves us with

$$\left(1.0 \times 10^{-4} + x\right)(2x)^2 = 2.5 \times 10^{-13}$$

To solve this equation for *x*, let's make the following assumption

$$\left[Pb^{2+}\right] = 1.0 \times 10^{-4} + x \approx 1.0 \times 10^{-4} \text{ M}$$

Solving for *x* gives its value as 2.50×10^{-5}; however, when we substitute this value for *x* back, we find that the calculated concentration of Pb^{2+} at equilibrium

$$\left[Pb^{2+}\right] = 1.0 \times 10^{-4} + x = 1.0 \times 10^{-4} + 2.50 \times 10^{-5} = 1.25 \times 10^{-4} \text{ M}$$

is 25% greater than our assumption of 1.0×10^{-4} M. This error is unreasonably large.

Rather than shouting in frustration, let's make a new assumption. Our first assumption—that the concentration of Pb^{2+} is 1.0×10^{-4} M—was too small. The calculated concentration of 1.25×10^{-4} M, therefore, probably is a too large, but closer to the correct concentration than was our first assumption. For our second approximation, let's assume that

$$\left[Pb^{2+}\right] = 1.0 \times 10^{-4} + x = 1.25 \times 10^{-4}\text{M}$$

Substituting into Equation 6.7.1 and solving for *x* gives its value as 2.24×10^{-5}. The resulting concentration of Pb^{2+} is

$$\left[Pb^{2+}\right] = 1.0 \times 10^{-4} + 2.24 \times 10^{-5} = 1.22 \times 10^{-4} \text{ M}$$

which differs from our assumption of 1.25×10^{-4} M by 2.4%. Because the original concentration of Pb^{2+} is given to two significant figure, this is a more reasonable error. Our final solution, to two significant figures, is

$$\left[Pb^{2+}\right] = 1.2 \times 10^{-4} \text{ M and } [IO_3] = 4.5 \times 10^{-5} \text{ M}$$

and the molar solubility of $Pb(IO_3)_2$ is 2.2×10^{-5} mol/L. This iterative approach to solving the problems is known as the ***method of successive approximations***.

? Exercise 6.7.2

Calculate the molar solubility for Hg_2Cl_2 in 0.10 M NaCl and compare your answer to its molar solubility in deionized water (see Exercise 6.7.1).

Answer

We begin by setting up a table to help us keep track of the concentrations Hg_2^{2+} and Cl^- as this system moves toward and reaches equilibrium.

concentration	Hg_2Cl_2 (*s*)	⇌	Hg_2^{2+} (*aq*)	+	Cl^- (*aq*)
initial	solid		0		0.10
change	solid		+*x*		+2*x*
equilibrium	solid		*x*		0.1 + 2*x*

Substituting the equilibrium concentrations into the K_{sp} expression for Hg_2Cl_2 leaves us with a difficult to solve cubic equation.

$$K_{sp} = \left[Hg_2^{2+}\right]\left[Cl^-\right]^2 = (x)(0.10 + 2x)^2 = 4x^3 + 0.40x^2 + 0.010x$$

Let's make an assumption to simplify this problem. Because we expect the value of *x* to be small, let's assume that

$$\left[Cl^-\right] = 0.10 + 2x \approx 0.10$$

This simplifies our problem to

$$K_{sp} = \left[Hg_2^{2+}\right]\left[Cl^-\right]^2 = (x)(0.10)^2 = 0.010x = 1.2 \times 10^{-18}$$

which gives the value of *x* as 1.2×10^{-16} M. The difference between the actual concentration of Cl^-, which is (0.10 + 2*x*) M, and our assumption that it is 0.10 M introduces an error of 2.4×10^{-13} %. This is a negligible error. The molar solubility of Hg_2Cl_2 is the same as the concentration of Hg_2^{2+}, or 1.2×10^{-16} M. As expected, the molar solubility in 0.10 M NaCl is less than 6.7×10^{-7} mol/L, which is its solubility in water (see solution to Exercise 6.7.1).

A Systematic Approach to Solving Equilibrium Problems

Calculating the solubility of $Pb(IO_3)_2$ in a solution of $Pb(NO_3)_2$ is more complicated than calculating its solubility in deionized water. The calculation, however, is still relatively easy to organize and the simplifying assumptions are fairly obvious. This problem is reasonably straightforward because it involves only one equilibrium reaction and one equilibrium constant.

Determining the equilibrium composition of a system with multiple equilibrium reactions is more complicated. In this section we introduce a systematic approach to setting-up and solving equilibrium problems. As shown in Table 6.7.1 , this approach involves four steps.

Table 6.7.1 . Systematic Approach to Solving Equilibrium Problems

Step 1

Write all relevant equilibrium reactions and equilibrium constant expressions.

Step 2

Count the unique species that appear in the equilibrium constant expressions; these are your unknowns. You have enough information to solve the problem if the number of unknowns equals the number of equilibrium constant expressions. If not, add a mass balance equation and/or a charge balance equation. Continue adding equations until the number of equations equals the number of unknowns.

Step 3

Combine your equations and solve for one unknown. Whenever possible, simplify the algebra by making appropriate assumptions. If you make an assumption, set a limit for its error. This decision influences your evaluation of the assumption.

Step 4

Check your assumptions. If any assumption proves invalid, return to the previous step and continue solving. The problem is complete when you have an answer that does not violate any of your assumptions.

In addition to equilibrium constant expressions, two other equations are important to this systematic approach to solving an equilibrium problem. The first of these equations is a ***mass balance equation***, which simply is a statement that matter is conserved during a chemical reaction. In a solution of acetic acid, for example, the combined concentrations of the conjugate weak acid, CH_3COOH, and the conjugate weak base, CH_3COO^-, must equal acetic acid's initial concentration, C_{CH_3COOH}.

$$C_{CH_3COOH} = \left[CH_3COOH\right] + \left[CH_3COO^-\right]$$

> You may recall from Chapter 2 that this is the difference between a formal concentration and a molar concentration. The variable *C* represents a formal concentration.

The second equation is a ***charge balance equation***, which requires that the total positive charge from the cations equal the total negative charge from the anions. Mathematically, the charge balance equation is

$$\sum_{i=1}^{n}(z^+)_i\left[C^{z+}\right]_i = -\sum_{j=1}^{m}(z^-)_j\left[A^{z-}\right]_j$$

where $[C^{z+}]_i$ and $[A^{z-}]_j$ are, respectively, the concentrations of the i^{th} cation and the j^{th} anion, and $(z^+)_i$ and $(z^-)_j$ are the charges for the i^{th} cation and the j^{th} anion. Every ion in solution, even if it does not appear in an equilibrium reaction, must appear in the charge balance equation. For example, the charge balance equation for an aqueous solution of $Ca(NO_3)_2$ is

$$2 \times \left[Ca^{2+}\right] + \left[H_3O^+\right] = \left[OH^+\right] + \left[NO_3^-\right]$$

Note that we multiply the concentration of Ca^{2+} by two and that we include the concentrations of H_3O^+ and OH^-.

A charge balance is a conservation of a charge. The minus sign in front of the summation term on the right side of the charge balance equation ensures that both summations are positive. There are situations where it is impossible to write a charge balance equation because we do not have enough information about the solution's composition. For example, suppose we fix a solution's pH using a buffer. If the buffer's composition is not specified, then we cannot write a charge balance equation.

✔ Example 6.7.2

Write mass balance equations and a charge balance equation for a 0.10 M solution of $NaHCO_3$.

Solution

It is easier to keep track of the species in solution if we write down the reactions that define the solution's composition. These reactions are the dissolution of a soluble salt

$$NaHCO_3(s) \rightarrow Na^+(aq) + HCO_3^-(aq)$$

and the acid–base dissociation reactions of HCO_3^- and H_2O

$$HCO_3^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CO_3^{2-}(aq)$$

$$HCO_3^-(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + H_2CO_3(aq)$$

$$2H_2O(l) \rightleftharpoons H_3O^+(aq) + OH^-(aq)$$

The mass balance equations are

$$0.10\text{M} = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$$

$$0.10\text{ M} = [Na^+]$$

and the charge balance equation is

$$[Na^+] + [H_3O^+] = [OH^-] + [HCO_3^-] + 2 \times [CO_3^{2-}]$$

? Exercise 6.7.3

Write appropriate mass balance and charge balance equations for a solution containing 0.10 M KH_2PO_4 and 0.050 M Na_2HPO_4.

Answer

To help us determine what ions are in solution, let's write down all the reaction needed to prepare the solutions and the equilibrium reactions that take place within these solutions. These reactions are the dissolution of two soluble salts

$$KH_2PO_4(s) \longrightarrow K^+(aq) + H_2PO_4^-(aq)$$

$$NaHPO_4(s) \longrightarrow Na^+(aq) + HPO_4^{2-}(aq)$$

and the acid–base dissociation reactions for $H_2PO_4^-$, HPO_4^{2-}. and H_2O.

$$H_2PO_4^-(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + HPO_4^{2-}(aq)$$

$$H_2PO_4^-(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + H_3PO_4(aq)$$

$$HPO_4^{2-}(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + PO_4^{3-}(aq)$$

$$2H_2O(l) \rightleftharpoons H_3O^+(aq) + OH^-(aq)$$

Note that we did not include the base dissociation reaction for HPO_4^{2-} because we already accounted for its product, $H_2PO_4^-$, in another reaction. The mass balance equations for K^+ and Na^+ are straightforward

$$[K^+] = 0.10\text{ M and } [Na^+] = 0.10\text{ M}$$

but the mass balance equation for phosphate takes a bit more thought. Both $H_2PO_4^-$ and HPO_4^{2-} produce the same ions in solution. We can, therefore, imagine that the solution initially contains 0.15 M KH_2PO_4, which gives the following mass balance equation.

$$[H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}] = 0.15\text{ M}$$

The charge balance equation is

$$[H_3O^+] + [K^+] + [Na^+] = [H_2PO_4^-] + 2 \times [HPO_4^{2-}] + 3 \times [PO_4^{3-}] + [OH^-]$$

pH of a Monoprotic Weak Acid

To illustrate the systematic approach to solving equilibrium problems, let's calculate the pH of 1.0 M HF. Two equilibrium reactions affect the pH. The first, and most obvious, is the acid dissociation reaction for HF

$$HF(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + F^-(aq)$$

for which the equilibrium constant expression is

$$K_a = \frac{[H_3O^+]\,[F^-]}{[HF]} = 6.8 \times 10^{-4} \tag{6.7.3}$$

The second equilibrium reaction is the dissociation of water, which is an obvious yet easily neglected reaction

$$2H_2O(l) \rightleftharpoons H_3O^+(aq) + OH^-(aq)$$

$$K_w = [H_3O^+]\,[OH^-] = 1.00 \times 10^{-14} \tag{6.7.4}$$

Counting unknowns, we find four: [HF], [F^-], [H_3O^+], and [OH^-]. To solve this problem we need two additional equations. These equations are a mass balance equation on hydrofluoric acid

$$C_{HF} = [HF] + [F^-] = 1.0\text{M} \tag{6.7.5}$$

and a charge balance equation

$$[H_3O^+] = [OH^-] + [F^-] \tag{6.7.6}$$

With four equations and four unknowns, we are ready to solve the problem. Before doing so, let's simplify the algebra by making two assumptions.

Assumption One. Because HF is a weak acid, we know that the solution is acidic. For an acidic solution it is reasonable to assume that

$$[H_3O^+] >> [OH^-]$$

which simplifies the charge balance equation to

$$[H_3O^+] = [F^-] \tag{6.7.7}$$

Assumption Two. Because HF is a weak acid, very little of it dissociates to form F^-. Most of the HF remains in its conjugate weak acid form and it is reasonable to assume that

$$[HF] >> [F^-]$$

which simplifies the mass balance equation to

$$C_{HF} = [HF] = 1.0\ M \tag{6.7.8}$$

For this exercise let's accept an assumption if it introduces an error of less than ±5%.

Substituting Equation 6.7.7 and Equation 6.7.8 into Equation 6.7.3, and solving for the concentration of H_3O^+ gives us

$$K_a = \frac{[H_3O^+][F^-]}{[HF]} = \frac{[H_3O^+][H_3O^+]}{C_{HF}} = \frac{[H_3O^+]^2}{C_{HF}} = 6.8 \times 10^{-4}$$

$$[H_3O^+] = \sqrt{K_a C_{HF}} = \sqrt{(6.8 \times 10^{-4})(1.0)} = 2.6 \times 10^{-2}$$

Before accepting this answer, we must verify our assumptions. The first assumption is that $[OH^-]$ is significantly smaller than $[H_3O^+]$. Using Equation 6.7.4, we find that

$$[OH^-] = \frac{K_w}{[H_3O^+]} = \frac{1.00 \times 10^{-14}}{2.6 \times 10^{-2}} = 3.8 \times 10^{-13}$$

Clearly this assumption is acceptable. The second assumption is that $[F^-]$ is significantly smaller than [HF]. From Equation 6.7.7 we have

$$[F^-] = 2.6 \times 10^{-2}\ M$$

Because $[F^-]$ is 2.60% of C_{HF}, this assumption also is acceptable. Given that $[H_3O^+]$ is 2.6×10^{-2} M, the pH of 1.0 M HF is 1.59.

How does the calculation change if we require that the error introduced in our assumptions be less than ±1%? In this case we no longer can assume that [HF] >> $[F^-]$ and we cannot simplify the mass balance equation. Solving the mass balance equation for [HF]

$$[HF] = C_{HF} - [F^-] = C_{HF} - [H_3O^+]$$

and substituting into the K_a expression along with Equation 6.7.7 gives

$$K_a = \frac{[H_3O^+]^2}{C_{HF} - [H_3O^+]}$$

Rearranging this equation leaves us with a quadratic equation

$$[H_3O^+]^2 + K_a[H_3O^+] - K_a C_{HF} = 0$$

which we solve using the quadratic formula

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where *a*, *b*, and *c* are the coefficients in the quadratic equation

$$ax^2 + bx + c = 0$$

Solving a quadratic equation gives two roots, only one of which has chemical significance. For our problem, the equation's roots are

$$x = \frac{-6.8 \times 10^{-4} \pm \sqrt{(6.8 \times 10^{-4})^2 - (4)(1)(-6.8 \times 10^{-4})}}{(2)(1)}$$

$$x = \frac{-6.8 \times 10^{-4} \pm 5.22 \times 10^{-2}}{2}$$

$$x = 2.57 \times 10^{-2} \text{ or } -2.64 \times 10^{-2}$$

Only the positive root is chemically significant because the negative root gives a negative concentration for H_3O^+. Thus, $[H_3O^+]$ is 2.57×10^{-2} M and the pH is 1.59.

You can extend this approach to calculating the pH of a monoprotic weak base by replacing K_a with K_b, replacing C_{HF} with the weak base's concentration, and solving for $[OH^-]$ in place of $[H_3O^+]$.

pH of a Polyprotic Acid or Base

A more challenging problem is to find the pH of a solution that contains a polyprotic weak acid or one of its conjugate species. As an example, consider the amino acid alanine, whose structure is shown in Figure 6.7.1 . The ladder diagram in Figure 6.7.2 shows alanine's three acid–base forms and their respective areas of predominance. For simplicity, we identify these species as H_2L^+, HL, and L^-.

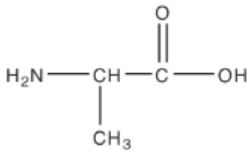


Figure 6.7.1 . Structure of the amino acid alanine, which has pK_a values of 2.348 (–COOH) and 9.867 ($–NH_2$).

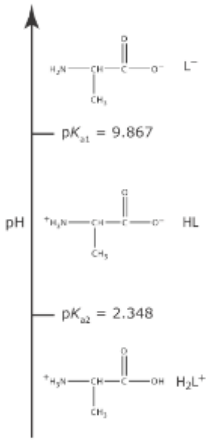


Figure 6.7.2 . Ladder diagram for alanine.

pH of 0.10 M Alanine Hydrochloride (H_2L^+)

Alanine hydrochloride is the salt of the diprotic weak acid H_2L^+ and Cl^-. Because H_2L^+ has two acid dissociation reactions, a complete systematic solution to this problem is more complicated than that for a monoprotic weak acid. The ladder diagram in Figure 6.7.2 helps us simplify the problem. Because the areas of predominance for H_2L^+ and L^- are so far apart, we can assume that a solution of H_2L^+ will not contain a significant amount of L^-. As a result, we can treat H_2L^+ as though it is a monoprotic weak acid. Calculating the pH of 0.10 M alanine hydrochloride, which is 1.72, is left to the reader as an exercise.

pH of 0.10 M Sodium Alaninate (L^-)

The alaninate ion is a diprotic weak base. Because L^- has two base dissociation reactions, a complete systematic solution to this problem is more complicated than that for a monoprotic weak base. Once again, the ladder diagram in Figure 6.7.2 helps us simplify the problem. Because the areas of predominance for H_2L^+ and L^- are so far apart, we can assume that a solution of L^- will not contain a significant amount of H_2L^+. As a result, we can treat L^- as though it is a monoprotic weak base. Calculating the pH of 0.10 M sodium alaninate, which is 11.42, is left to the reader as an exercise.

pH of 0.10 M Alanine (HL)

Finding the pH of a solution of alanine is more complicated than our previous two examples because we cannot ignore the presence of either H_2L^+ or L^-. To calculate the solution's pH we must consider alanine's acid dissociation reaction

$$\mathrm{HL}(aq) + \mathrm{H_2O}(l) \rightleftharpoons \mathrm{H_3O^+}(aq) + \mathrm{L^-}(aq)$$

and its base dissociation reaction

$$\mathrm{HL}(aq) + \mathrm{H_2O}(l) \rightleftharpoons \mathrm{OH^-}(aq) + \mathrm{H_2L^+}(aq)$$

and, as always, we must also consider the dissociation of water

$$2\mathrm{H_2O}(l) \rightleftharpoons \mathrm{H_3O^+}(aq) + \mathrm{OH^-}(aq)$$

This leaves us with five unknowns—$[H_2L^+]$, [HL], $[L^-]$, $[H_3O^+]$, and $[OH^-]$—for which we need five equations. These equations are K_{a2} and K_{b2} for alanine

$$K_{a2} = \frac{[\mathrm{H_3O^+}]\,[\mathrm{L^-}]}{[\mathrm{HL}]}$$

$$K_{b2} = \frac{K_w}{K_{a1}} = \frac{[\mathrm{OH^-}]\,[\mathrm{H_2L^+}]}{[\mathrm{HL}]}$$

the K_w equation

$$K_w = [\mathrm{H_3O^+}]\,[\mathrm{OH^-}]$$

a mass balance equation for alanine

$$C_{\mathrm{HL}} = [\mathrm{H_2L^+}] + [\mathrm{HL}] + [\mathrm{L^-}]$$

and a charge balance equation

$$[\mathrm{H_2L^+}] + [\mathrm{H_3O^+}] = [\mathrm{OH^-}] + [\mathrm{L^-}]$$

Because HL is a weak acid and a weak base, it seems reasonable to assume that little of it will dissociate and that

$$[\mathrm{HL}] >> [\mathrm{H_2L^+}] + [\mathrm{L^-}]$$

which allows us to simplify the mass balance equation to

$$C_{\mathrm{HL}} = [\mathrm{HL}]$$

Next we solve K_{b2} for $[H_2L^+]$

$$[\mathrm{H_2L^+}] = \frac{K_w[\mathrm{HL}]}{K_{a1}\,[\mathrm{OH^-}]} = \frac{[\mathrm{H_3O^+}]\,[\mathrm{HL}]}{K_{a1}} = \frac{C_{\mathrm{HL}}\,[\mathrm{H_3O^+}]}{K_{a1}}$$

and solve K_{a2} for $[L^-]$

$$[\mathrm{L^-}] = \frac{K_{a2}[\mathrm{HL}]}{[\mathrm{H_3O^+}]} = \frac{K_{a2}C_{\mathrm{HL}}}{[\mathrm{H_3O^+}]}$$

Substituting these equations for $[H_2L^+]$ and $[L^-]$, and the equation for K_w, into the charge balance equation give us

$$\frac{C_{\mathrm{HL}}\,[\mathrm{H_3O^+}]}{K_{a1}} + [\mathrm{H_3O^+}] = \frac{K_w}{[\mathrm{H_3O^+}]} + \frac{K_{a2}C_{\mathrm{HL}}}{[\mathrm{H_3O^+}]}$$

which we simplify to

$$[H_3O^+]\left(\frac{C_{HL}}{K_{a1}}+1\right)=\frac{1}{[H_3O^+]}(K_w+K_{a2}C_{HL})$$

$$[H_3O^+]^2=\frac{(K_{a2}C_{HL}+K_w)}{\frac{C_{HL}}{K_{a1}}+1}=\frac{K_{a1}\,(K_{a2}C_{HL}+K_w)}{C_{HL}+K_{a1}}$$

$$[H_3O^+]=\sqrt{\frac{(K_{a1}\,K_{a2}C_{HL}+K_{a1}\,K_w)}{C_{HL}+K_{a1}}}$$

We can further simplify this equation if $K_{a1}K_w << K_{a1}K_{a2}C_{HL}$, and if $K_{a1} << C_{HL}$, leaving us with

$$[H_3O^+]=\sqrt{K_{a1}\,K_{a2}}$$

For a solution of 0.10 M alanine the $[H_3O^+]$ is

$$[H_3O^+]=\sqrt{(4.487\times10^{-3})\,(1.358\times10^{-10})}=7.806\times10^{-7}\ \text{M}$$

or a pH of 6.11.

? Exercise 6.7.5

Verify that each assumption in our solution for the pH of 0.10 M alanine is reasonable, using ±5% as the limit for the acceptable error.

Answer

Effect of Complexation on Solubility

One method for increasing a precipitate's solubility is to add a ligand that forms soluble complexes with one of the precipitate's ions. For example, the solubility of AgI increases in the presence of NH_3 due to the formation of the soluble $Ag(NH_3)_2^+$ complex. As a final illustration of the systematic approach to solving equilibrium problems, let's calculate the molar solubility of AgI in 0.10 M NH_3.

We begin by writing the relevant equilibrium reactions, which includes the solubility of AgI, the acid–base chemistry of NH_3 and H_2O, and the metal-ligand complexation chemistry between Ag^+ and NH_3.

$$AgI(s)\rightleftharpoons Ag^+(aq)+I^-(aq)$$
$$NH_3(aq)+H_2O(l)\rightleftharpoons OH^-(aq)+NH_4^+(aq)$$
$$2H_2O(l)\rightleftharpoons H_3O^+(aq)+OH^-(aq)$$
$$Ag^+(aq)+2NH_3(aq)\rightleftharpoons Ag(NH_3)_2^+(aq)$$

This leaves us with seven unknowns—$[Ag^+]$, $[I^-]$, $[NH_3]$, $[NH_4^+]$, $[OH^-]$, $[H_3O^+]$, and $[Ag(NH_3)_2^+]$—and a need for seven equations. Four of the equations we need to solve this problem are the equilibrium constant expressions

$$K_{sp}=[Ag^+]\,[I^-]=8.3\times10^{-17} \tag{6.7.9}$$

$$K_b=\frac{[NH_4^+]\,[OH^-]}{[NH_3]}=1.75\times10^{-5} \tag{6.7.10}$$

$$K_w=[H_3O^+]\,[OH^-]=1.00\times10^{-14} \tag{6.7.11}$$

$$\beta_2=\frac{[Ag(NH_3)_2^+]}{[Ag^+]\,[NH_3]^2}=1.7\times10^7 \tag{6.7.12}$$

We still need three additional equations. The first of these equations is a mass balance for NH_3.

$$C_{NH_3}=[NH_3]+[NH_4^+]+2\times[Ag(NH_3)_2^+] \tag{6.7.13}$$

In writing this mass balance equation we multiply the concentration of $Ag(NH_3)_2^+$ by two since there are two moles of NH_3 per mole of $Ag(NH_3)_2^+$. The second additional equation is a mass balance between iodide and silver. Because AgI is the only source of I^- and Ag^+, each iodide in solution must have an associated silver ion, which may be Ag^+ or $Ag(NH_3)_2^+$; thus

$$[I^-]=[Ag^+]+[Ag(NH_3)_2^+] \tag{6.7.14}$$

Finally, we include a charge balance equation.

$$[Ag^+]+[Ag(NH_3)_2^+]+[NH_4^+]+[H_3O^+]=[OH^-]+[I^-] \tag{6.7.15}$$

Although the problem looks challenging, three assumptions greatly simplify the algebra.

Assumption One. Because the formation of the $Ag(NH_3)_2^+$ complex is so favorable (β_2 is 1.7×10^7), there is very little free Ag^+ in solution and it is reasonable to assume that

$$[Ag^+] << [Ag(NH_3)_2^+]$$

Assumption Two. Because NH_3 is a weak base we may reasonably assume that most uncomplexed ammonia remains as NH_3; thus

$$[NH_4^+] << [NH_3]$$

Assumption Three. Because K_{sp} for AgI is significantly smaller than β_2 for $Ag(NH_3)_2^+$, the solubility of AgI probably is small enough that very little ammonia is needed to form the metal–ligand complex; thus

$$[Ag(NH_3)_2^+] << [NH_3]$$

As we use these assumptions to simplify the algebra, let's set ±5% as the limit for error.

Assumption two and assumption three suggest that the concentration of NH_3 is much larger than the concentrations of either NH_4^+ or $Ag(NH_3)_2^+$, which allows us to simplify the mass balance equation for NH_3 to

$$C_{NH_3} = [NH_3] \tag{6.7.16}$$

Finally, using assumption one, which suggests that the concentration of $Ag(NH_3)_2^+$ is much larger than the concentration of Ag^+, we simplify the mass balance equation for I^- to

$$[I^-] = [Ag(NH_3)_2^+] \tag{6.7.17}$$

Now we are ready to combine equations and to solve the problem. We begin by solving Equation 6.7.9 for $[Ag^+]$ and substitute it into β_2 (Equation 6.7.12), which leaves us with

$$\beta_2 = \frac{[Ag(NH_3)_2^+]\,[I^-]}{K_{sp}[NH_3]^2} \tag{6.7.18}$$

Next we substitute Equation 6.7.16 and Equation 6.7.17 into Equation 6.7.18, obtaining

$$\beta_2 = \frac{[I^-]^2}{K_{sp}(C_{NH_3})^2} \tag{6.7.19}$$

Solving Equation 6.7.19 for $[I^-]$ gives

$$[I^-] = C_{NH_3}\sqrt{\beta_2 K_{sp}} =$$

$$(0.10)\sqrt{(1.7\times10^7)\,(8.3\times10^{-17})} = 3.76\times10^{-6}\ \text{M}$$

Because one mole of AgI produces one mole of I^-, the molar solubility of AgI is the same as the $[I^-]$, or 3.8×10^{-6} mol/L.

Before we accept this answer we need to check our assumptions. Substituting $[I^-]$ into Equation 6.7.9, we find that the concentration of Ag^+ is

$$[Ag^+] = \frac{K_p}{[I^-]} = \frac{8.3\times10^{-17}}{3.76\times10^{-6}} = 2.2\times10^{-11}\ \text{M}$$

Substituting the concentrations of I^- and Ag+ into the mass balance equation for iodide (Equation 6.7.14), gives the concentration of $Ag(NH_3)_2^+$ as

$$[Ag(NH_3)_2^+] = [I^-] - [Ag^+] = 3.76\times10^{-6} - 2.2\times10^{-11} = 3.76\times10^{-6}\ \text{M}$$

Our first assumption that $[Ag^+]$ is significantly smaller than the $[Ag(NH_3)_2^+]$ is reasonable.

Substituting the concentrations of Ag^+ and $Ag(NH_3)_2^+$ into Equation 6.7.12 and solving for $[NH_3]$, gives

$$[NH_3] = \sqrt{\frac{[Ag(NH_3)_2^+]}{[Ag^+]\,\beta_2}} = \sqrt{\frac{3.76\times10^{-6}}{(2.2\times10^{-11})\,(1.7\times10^7)}} = 0.10\ \text{M}$$

From the mass balance equation for NH3 (Equation 6.7.12) we see that $[NH_4^+]$ is negligible, verifying our second assumption that $[NH_4^+]$ is significantly smaller than $[NH_3]$. Our third assumption that $[Ag(NH_3)_2^+]$ is significantly smaller than $[NH_3]$ also is reasonable.

> Did you notice that our solution to this problem did not make use of Equation 6.7.15, the charge balance equation? The reason for this is that we did not try to solve for the concentration of all seven species. If we need to know the reaction mixture's complete composition at equilibrium, then we will need to incorporate the charge balance equation into our solution.

6.8: Buffer Solutions

Adding as little as 0.1 mL of concentrated HCl to a liter of H_2O shifts the pH from 7.0 to 3.0. Adding the same amount of HCl to a liter of a solution that 0.1 M in acetic acid and 0.1 M in sodium acetate, however, results in a negligible change in pH. Why do these two solutions respond so differently to the addition of HCl?

A mixture of acetic acid and sodium acetate is one example of an acid–base ***buffer***. To understand how this buffer works to limit the change in pH, we need to consider its acid dissociation reaction

$$CH_3COOH(aq) + H_2O(l) \rightleftharpoons H_3O^+(aq) + CH_3COO^-(aq)$$

and its corresponding acid dissociation constant

$$K_a = \frac{[CH_3COO^-]\,[H_3O^+]}{[CH_3COOH]} = 1.75\times10^{-5} \tag{6.8.1}$$

Taking the negative log of the terms in Equation 6.8.1 and solving for pH leaves us with the result shown here.

$$pH = pK_a + \log\frac{[CH_3COO^-]}{[CH_3COOH]}$$

$$pH = 4.76 + \log\frac{[CH_3COO^-]}{[CH_3COOH]} \tag{6.8.2}$$

> You may recall that we developed these same equations in Chapter 6.6 when we introduced ladder diagrams.

Buffering occurs because of the logarithmic relationship between pH and the concentration ratio of acetate and acetic acid. Here is an example to illustrate this point. If the concentrations of acetic acid and acetate are equal, the buffer's pH is 4.76. If we convert 10% of the acetate to acetic acid, by adding a strong acid, the ratio $[CH_3COO^-]/[CH_3COOH]$ changes from 1.00 to 0.818, and the pH decreases from 4.76 to 4.67—a decrease of only 0.09 pH units.

> The ratio $[CH_3COO^-]/[CH_3COOH]$ becomes 0.9/1.1 = 0.818 and the pH becomes
>
> $$\text{pH} = 4.76 + \log(0.818) = 4.67$$

Systematic Solution to Buffer Problems

Equation 6.8.2 is written in terms of the equilibrium concentrations of CH_3COOH and of CH_3COO^-. A more useful relationship relates a buffer's pH to the initial concentrations of the weak acid and the weak base. We can derive a general buffer equation by considering the following reactions for a weak acid, HA, and the soluble salt of its conjugate weak base, NaA.

$$\text{NaA}(s) \rightarrow \text{Na}^+(aq) + \text{A}^-(aq)$$
$$\text{HA}(aq) + \text{H}_2\text{O}(l) \rightleftharpoons \text{H}_3\text{O}^+(aq) + \text{A}^-(aq)$$
$$2\text{H}_2\text{O}(l) \rightleftharpoons \text{H}_3\text{O}^+(aq) + \text{OH}^-(aq)$$

Because the concentrations of Na^+, A^-, HA, H_3O^+, and OH^- are unknown, we need five equations to define the solution's composition. Two of these equations are the equilibrium constant expressions for HA and H_2O.

$$K_a = \frac{[\text{H}_3\text{O}^+]\,[\text{A}^-]}{[\text{HA}]} \tag{6.8.3}$$

$$K_w = [\text{H}_3\text{O}^+]\,[\text{OH}^-]$$

The remaining three equations are mass balance equations for HA and Na^+

$$C_{\text{HA}} + C_{\text{NaA}} = [\text{HA}] + [\text{A}^-] \tag{6.8.4}$$

$$C_{\text{NaA}} = [\text{Na}^+] \tag{6.8.5}$$

and a charge balance equation

$$[\text{H}_3\text{O}^+] + [\text{Na}^+] = [\text{OH}^-] + [\text{A}^-] \tag{6.8.6}$$

Substituting Equation 6.8.5 into Equation 6.8.6 and solving for $[A^-]$ gives

$$[\text{A}^-] = C_{\text{NaA}} - [\text{OH}^-] + [\text{H}_3\text{O}^+] \tag{6.8.7}$$

Next, we substitute Equation 6.8.7 into Equation 6.8.4, which gives the concentration of HA as

$$[\text{HA}] = C_{\text{HA}} + [\text{OH}^-] - [\text{H}_3\text{O}^+] \tag{6.8.8}$$

Finally, we substitute Equation 6.8.7 and Equation 6.8.8 into Equation 6.8.3 and solve for pH to arrive at a general equation for a buffer's pH.

$$\text{pH} = \text{p}K_a + \log\frac{C_{\text{NaA}} - [\text{OH}^-] + [\text{H}_3\text{O}^+]}{C_{\text{HA}} + [\text{OH}^-] - [\text{H}_3\text{O}^+]}$$

If the initial concentrations of the weak acid, C_{HA}, and the weak base, C_{NaA}, are significantly greater than $[H_3O^+]$ and $[OH^-]$, then we can simplify the general equation to the ***Henderson–Hasselbalch equation***.

$$\text{pH} = \text{p}K_a + \log\frac{C_{\text{NaA}}}{C_{\text{HA}}} \tag{6.8.9}$$

As outlined below, the Henderson–Hasselbalch equation provides a simple way to calculate the pH of a buffer, and to determine the change in pH upon adding a strong acid or strong base.

> Lawrence Henderson (1878-1942) first developed a relationship between $[H_3O^+]$, [HA], and $[A^-]$ while studying the buffering of blood. Kurt Hasselbalch (1874-1962) modified Henderson's equation by transforming it to the logarithmic form shown in Equation 6.8.9. The assumptions that lead to Equation 6.8.9 result in a minimal error in pH (<±5%) for larger concentrations of HA and A^-, for concentrations of HA and A^- that are similar in magnitude, and for weak acid's with pK_a values closer to 7. For most problems in this textbook, Equation 6.8.9 provides acceptable results. Be sure, however, to test your assumptions. For a discussion of the Henderson–Hasselbalch equation, including the error inherent in Equation 6.8.9, see Po, H. N.; Senozan, N. M. "The Henderson–Hasselbalch Equation: Its History and Limitations," *J. Chem. Educ.* **2001**, *78*, 1499–1503.

✔ Example 6.8.1

Calculate the pH of a buffer that is 0.020 M in NH_3 and 0.030 M in NH_4Cl. What is the pH after we add 1.0 mL of 0.10 M NaOH to 0.10 L of this buffer?

Solution

The acid dissociation constant for NH_4^+ is 5.70×10^{-10}, which is a pK_a of 9.24. Substituting the initial concentrations of NH_3 and NH_4Cl into Equation 6.8.9 and solving, we find that the buffer's pH is

$$\text{pH} = 9.24 + \log\frac{0.020}{0.030} = 9.06$$

With a pH of 9.06, the concentration of H_3O^+ is 8.71×10^{-10} and the concentration of OH^- is 1.15×10^{-5}. Because both of these concentrations are much smaller than either C_{NH_3} or C_{NH_4Cl}, the approximations used to derive Equation 6.8.9 are reasonable.

Adding NaOH converts a portion of the NH_4^+ to NH_3 following reaction

$$\text{NH}_4^+(aq) + \text{OH}^-(aq) \rightleftharpoons \text{H}_2\text{O}(l) + \text{NH}_3(aq)$$

Because this reaction's equilibrium constant is so large (it is equal to $(K_b)^{-1}$ or 5.7×10^4), we may treat the reaction as if it goes to completion. The new concentrations of NH_4^+ and NH_3 are

$$C_{\text{NH}_4^+} = \frac{\text{mol NH}_4^+ - \text{mol}\,\text{OH}^-}{V_{\text{total}}}$$

$$C_{\text{NH}_4^+} = \frac{(0.030\ \text{M})(0.10\ \text{L}) - (0.10\ \text{M})\,(1.0 \times 10^{-3}\ \text{L})}{0.10\ \text{L} + 1.0 \times 10^{-3}\ \text{L}} = 0.029\ \text{M}$$

$$C_{NH_3} = \frac{\text{mol } NH_3 + \text{mol } OH^-}{V_{total}}$$

$$C_{NH_3} = \frac{(0.020\text{ M})(0.10\text{ L}) + (0.10\text{ M})\left(1.0 \times 10^{-3}\text{ L}\right)}{0.10\text{ L} + 1.0 \times 10^{-3}\text{ L}} = 0.021\text{ M}$$

Substituting these concentrations into the equation 6.60 gives a pH of

$$\text{pH} = 9.24 + \log\frac{0.021}{0.029} = 9.10$$

Note that adding NaOH increases the pH from 9.06 to 9.10. As we expect, adding a base makes the pH more basic. Checking to see that the pH changes in the right direction is one way to catch a calculation error.

? Exercise 6.8.1

Calculate the pH of a buffer that is 0.10 M in KH_2PO_4 and 0.050 M in Na_2HPO_4. What is the pH after we add 5.0 mL of 0.20 M HCl to 0.10 L of this buffer. Use Appendix 11 to find the appropriate K_a value.

Answer

We can use a multiprotic weak acid to prepare buffers at as many different pH's as there are acidic protons, with the Henderson–Hasselbalch equation applying in each case. For example, for malonic acid (pK_{a1} = 2.85 and pK_{a2} = 5.70) we can prepare buffers with pH values of

$$\text{pH} = 2.85 + \log\frac{C_{HM^-}}{C_{H_2M}}$$

$$\text{pH} = 5.70 + \log\frac{C_{M^{2-}}}{C_{HM^-}}$$

where H_2M, HM^- and M^{2-} are malonic acid's different acid–base forms.

Although our treatment of buffers is based on acid–base chemistry, we can extend buffers to equilibria that involve complexation or redox reactions. For example, the Nernst equation for a solution that contains Fe^{2+} and Fe^{3+} is similar in form to the Henderson-Hasselbalch equation.

$$E = E^\circ_{Fe^{3+}/Fe^{2+}} - 0.05916\log\frac{[Fe^{2+}]}{[Fe^{3+}]}$$

A solution that contains similar concentrations of Fe^{2+} and Fe^{3+} is buffered to a potential near the standard state reduction potential for Fe^{3+}. We call such solutions redox buffers. Adding a strong oxidizing agent or a strong reducing agent to a redox buffer results in a small change in potential.

Representing Buffer Solutions with Ladder Diagrams

A ladder diagram provides a simple way to visualize a solution's predominate species as a function of solution conditions. It also provides a convenient way to show the range of solution conditions over which a buffer is effective. For example, an acid–base buffer exists when the concentrations of the weak acid and its conjugate weak base are similar. For convenience, let's assume that an acid–base buffer exists when

$$\frac{1}{10} \le \frac{[CH_3COO^-]}{[CH_3COOH]} \le \frac{10}{1}$$

Substituting these ratios into the Henderson–Hasselbalch equation

$$\text{pH} = \text{p}K_a + \log\frac{1}{10} = \text{p}K_a - 1$$

$$\text{pH} = \text{p}K_a + \log\frac{10}{1} = \text{p}K_a + 1$$

shows that an acid–base buffer works over a pH range of $pK_a \pm 1$.

Using the same approach, it is easy to show that a metal-ligand complexation buffer for ML_n exists when

$$\text{pL} = \log K_n \pm 1 \text{ or } \text{pL} = \log\beta_n \pm \frac{1}{n}$$

where K_n or β_n is the relevant stepwise or overall formation constant. For an oxidizing agent and its conjugate reducing agent, a redox buffer exists when

$$E = E^\circ \pm \frac{1}{n} \times \frac{RT}{F^\circ} = E^\circ \pm \frac{0.05916}{n}(\text{at } 25^\circ\text{C})$$

Figure 6.8.1 shows ladder diagrams with buffer regions for several equilibrium systems.

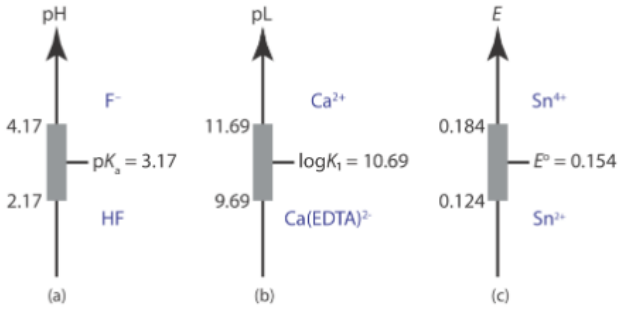


Figure 6.8.1 . Ladder diagrams showing buffer regions shaded in grey for (a) an acid–base buffer of HF and F^-; (b) a metal–ligand complexation buffer of Ca^{2+} and $Ca(EDTA)^{2-}$; and (c) an oxidation–reduction (redox) buffer of Sn^{4+} and Sn^{2+}.

Preparing a Buffer

Buffer capacity is the ability of a buffer to resist a change in pH when we add to it a strong acid or a strong base. A buffer's capacity to resist a change in pH is a function of the concentrations of the weak acid and the weak base, as well as their relative proportions. The importance of the weak acid's concentration and the weak base's concentration is obvious. The more moles of weak acid and weak base a buffer has, the more strong base or strong acid it can neutralize without a significant change in its pH.

> Although a higher concentration of buffering agents provides greater buffer capacity, there are reasons for using smaller concentrations, including the formation of unwanted precipitates and the tolerance of biological systems for high concentrations of dissolved salts.

The relative proportions of a weak acid and a weak base also affects how much the pH changes when we add a strong acid or a strong base. A buffer that is equimolar in weak acid and weak base requires a greater amount of strong acid or strong base to bring about a one unit change in pH. Consequently, a buffer is most effective against the addition of strong acids or strong bases when its pH is near the weak acid's pK_a value.

Buffer solutions are often prepared using standard "recipes" found in the chemical literature [see, for example, (a) Bower, V. E.; Bates, R. G. *J. Res. Natl. Bur. Stand. (U. S.)* **1955**, *55*, 197– 200; (b) Bates, R. G. *Ann. N. Y. Acad. Sci.* **1961**, *92*, 341–356; (c) Bates, R. G. *Determination of pH*, 2nd ed.; Wiley-Interscience: New York, 1973]. In addition, there are computer programs and on-line calculators to aid in preparing buffers [(a) Lambert, W. J. J. Chem. Educ. 1990, 67, 150–153; (b) http://www.bioinformatics.org/JaMBW/5/4/index.html.]. Perhaps the simplest way to make a buffer, however, is to prepare a solution that contains an appropriate conjugate weak acid and weak base, measure its pH, and then adjust the pH to the desired value by adding small portions of either a strong acid or a strong base.

> A good "rule of thumb" when choosing a buffer is to select one whose reagents have a pK_a value close to your desired pH.

7.1: The Importance of Sampling

When a manufacturer lists a chemical as ACS Reagent Grade, they must demonstrate that it conforms to specifications set by the American Chemical Society (ACS). For example, the ACS specifications for commercial NaBr require that the concentration of iron is less than 5 ppm. To verify that a production lot meets this standard, the manufacturer collects and analyzes several samples, reporting the average result on the product's label (Figure 7.1.1).

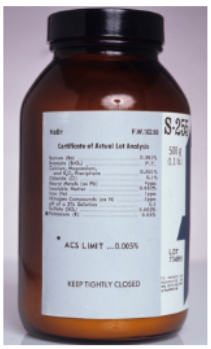


Figure 7.1.1 . Certificate of analysis for a production lot of NaBr. The result for iron meets the ACS specifications, but the result for potassium does not.

If the individual samples do not represent accurately the population from which they are drawn—a population that we call the ***target population***—then even a careful analysis will yield an inaccurate result. Extrapolating a result from a sample to its target population always introduces a determinate sampling error. To minimize this determinate sampling error, we must collect the right sample.

Even if we collect the right sample, indeterminate sampling errors may limit the usefulness of our analysis. Equation 7.1.1 shows that a confidence interval about the mean, $\overline{X}$, is proportional to the standard deviation, s, of the analysis

$$\mu = \overline{X} \pm \frac{ts}{\sqrt{n}} \tag{7.1.1}$$

where n is the number of samples and t is a statistical factor that accounts for the probability that the confidence interval contains the true value, μ.

> Equation 7.1.1 should be familiar to you. See Chapter 4 to review confidence intervals and see Appendix 4 for values of t.

Each step of an analysis contributes random error that affects the overall standard deviation. For convenience, let's divide an analysis into two steps—collecting the samples and analyzing the samples—each of which is characterized by a variance. Using a propagation of uncertainty, the relationship between the overall variance, s^2, and the variances due to sampling, s^2_{samp}, and the variance due to the analytical method, s^2_{meth}, is

> Although Equation 7.1.1 is written in terms of a standard deviation, s, a propagation of uncertainty is written in terms of variances, s^2. In this section, and those that follow, we will use both standard deviations and variances to discuss sampling uncertainty. For a review of the propagation of uncertainty, see Chapter 4.3 and Appendix 2.

Equation 7.1.2 shows that the overall variance for an analysis is limited by either the analytical method or sampling, or by both. Unfortunately, analysts often try to minimize the overall variance by improving only the method's precision. This is a futile effort, however, if the standard deviation for sampling is more than three times greater than that for the method [Youden, W. J. *J. Assoc. Off. Anal. Chem.* **1981**, *50*, 1007–1013]. Figure 7.1.2 shows how the ratio s_{samp}/s_{meth} affects the method's contribution to the overall variance. As shown by the dashed line, if the sample's standard deviation is $3\times$ the method's standard deviation, then indeterminate method errors explain only 10% of the overall variance. If indeterminate sampling errors are significant, decreasing s_{meth} provides only limited improvement in the overall precision.

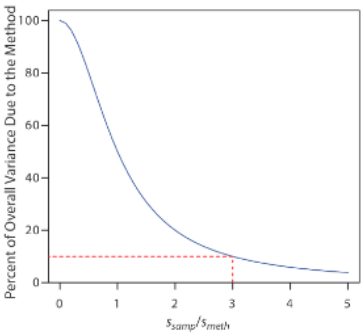


Figure 7.1.2 . The blue curve shows the method's contribution to the overall variance, s^2, as a function of the relative magnitude of the standard deviation in sampling, s_{samp}, and the method's standard deviation, s_{meth}. The dashed red line shows that the method accounts for only 10% of the overall variance when $s_{samp}/s_{meth} = 3 \times s_{meth}$. Understanding the relative importance of potential sources of indeterminate error is important when we consider how to improve the overall precision of the analysis.

✔ Example 7.1.1

A quantitative analysis gives a mean concentration of 12.6 ppm for an analyte. The method's standard deviation is 1.1 ppm and the standard deviation for sampling is 2.1 ppm. (a) What is the overall variance for the analysis? (b) By how much does the overall variance change if we improve s_{meth} by 10% to 0.99 ppm? (c) By how much does the overall variance change if we improve s_{samp} by 10% to 1.9 ppm?

Solution

(a) The overall variance is

$$s^2 = s^2_{samp} + s^2_{meth} = (2.1\ \text{ppm})^2 + (1.1\ \text{ppm})^2 = 5.6\ \text{ppm}^2$$

(b) Improving the method's standard deviation changes the overall variance to

$$s^2 = (2.1\ \text{ppm})^2 + (0.99\ \text{ppm})^2 = 5.4\ \text{ppm}^2$$

Improving the method's standard deviation by 10% improves the overall variance by approximately 4%.

(c) Changing the standard deviation for sampling

$$s^2 = (1.9\ \text{ppm})^2 + (1.1\ \text{ppm})^2 = 4.8\ \text{ppm}^2$$

improves the overall variance by almost 15%. As expected, because s_{samp} is larger than s_{meth}, we achieve a bigger improvement in the overall variance when we focus our attention on sampling problems.

To determine which step has the greatest effect on the overall variance, we need to measure both s_{samp} and s_{meth}. The analysis of replicate samples provides an estimate of the overall variance. To determine the method's variance we must analyze samples under conditions where we can assume that the sampling variance is negligible; the sampling variance is then determined by difference.

> There are several ways to minimize the standard deviation for sampling. Here are two examples. One approach is to use a standard reference material (SRM) that has been carefully prepared to minimize indeterminate sampling errors. When the sample is homogeneous—as is the case, for example, with an aqueous sample—then another useful approach is to conduct replicate analyses on a single sample.

✔ Example 7.1.2

The following data were collected as part of a study to determine the effect of sampling variance on the analysis of drug-animal feed formulations [Fricke, G. H.; Mischler, P. G.; Staffieri, F. P.; Houmyer, C. L. *Anal. Chem.* **1987**, *59*, 1213– 1217].

% drug (w/w)			% drug (w/w)		
0.0114	0.0099	0.0105	0.0105	0.0109	0.0107
0.0102	0.0106	0.0087	0.0103	0.0103	0.0104
0.0100	0.0095	0.0098	0.0101	0.0101	0.013
0.0105	0.0095	0.0097			

The data on the left were obtained under conditions where both s_{samp} and s_{meth} contribute to the overall variance. The data on the right were obtained under conditions where s_{samp} is insignificant. Determine the overall variance, and the standard deviations due to sampling and the analytical method. To which source of indeterminate error—sampling or the method—should we turn our attention if we want to improve the precision of the analysis?

Solution

Using the data on the left, the overall variance, s^2, is 4.71×10^{-7}. To find the method's contribution to the overall variance, s^2_{meth}, we use the data on the right, obtaining a value of 7.00×10^{-8}. The variance due to sampling, s^2_{samp}, is

$$s^2_{samp} = s^2 - s^2_{meth} = 4.71 \times 10^{-7} - 7.00 \times 10^{-8} = 4.01 \times 10^{-7}$$

Converting variances to standard deviations gives s_{samp} as 6.33×10^{-4} and s_{meth} as 2.65×10^{-4}. Because s_{samp} is more than twice as large as s_{meth}, improving the precision of the sampling process will have the greatest impact on the overall precision.

7.2: Designing a Sampling Plan

A sampling plan must support the goals of an analysis. For example, a material scientist interested in characterizing a metal's surface chemistry is more likely to choose a freshly exposed surface, created by cleaving the sample under vacuum, than a surface previously exposed to the atmosphere. In a qualitative analysis, a sample need not be identical to the original substance provided there is sufficient analyte present to ensure its detection. In fact, if the goal of an analysis is to identify a trace-level component, it may be desirable to discriminate against major components when collecting samples.

> For an interesting discussion of the importance of a sampling plan, see Buger, J. et al. "Do Scientists and Fishermen Collect the Same Size Fish? Possible Implications for Exposure Assessment," *Environ. Res.* **2006**, *101*, 34–41.

For a quantitative analysis, the sample's composition must represent accurately the target population, a requirement that necessitates a careful sampling plan. Among the issues we need to consider are these five questions.

1. From where within the target population should we collect samples?
2. What type of samples should we collect?
3. What is the minimum amount of sample needed for each analysis?
4. How many samples should we analyze?
5. How can we minimize the overall variance for the analysis?

Where to Sample the Target Population

A sampling error occurs whenever a sample's composition is not identical to its target population. If the target population is ***homogeneous***, then we can collect individual samples without giving consideration to where we collect sample. Unfortunately, in most situations the target population is ***heterogeneous*** and attention to where we collect samples is important. For example, due to settling a medication available as an oral suspension may have a higher concentration of its active ingredients at the bottom of the container. The composition of a clinical sample, such as blood or urine, may depend on when it is collected. A patient's blood glucose level, for instance, will change in response to eating and exercise. Other target populations show both a spatial and a temporal heterogeneity. The concentration of dissolved O_2 in a lake is heterogeneous due both to a change in seasons and to point sources of pollution.

> The composition of a homogeneous target population is the same regardless of where we sample, when we sample, or the size of our sample. For a heterogeneous target population, the composition is not the same at different locations, at different times, or for different sample sizes.

If the analyte's distribution within the target population is a concern, then our sampling plan must take this into account. When feasible, homogenizing the target population is a simple solution, although this often is impracticable. In addition, homogenizing a sample destroys information about the analyte's spatial or temporal distribution within the target population, information that may be of importance.

Random Sampling

The ideal sampling plan provides an unbiased estimate of the target population's properties. A ***random sampling*** is the easiest way to satisfy this requirement [Cohen, R. D. *J. Chem. Educ.* **1991**, *68*, 902–903]. Despite its apparent simplicity, a truly random sample is difficult to collect. Haphazard sampling, in which samples are collected without a sampling plan, is not random and may reflect an analyst's unintentional biases.

Here is a simple method to ensure that we collect random samples. First, we divide the target population into equal units and assign to each unit a unique number. Then, we use a random number table to select the units to sample. Example 7.2.1 provides an illustrative example. Appendix 14 provides a random number table that you can use to design a sampling plan.

Judgmental Sampling

The opposite of random sampling is selective, or ***judgmental sampling*** in which we use prior information about the target population to help guide our selection of samples. Judgmental sampling is more biased than random sampling, but requires fewer samples. Judgmental sampling is useful if we wish to limit the number of independent variables that might affect our results. For example, if we are studying the bioaccumulation of PCB's in fish, we may choose to exclude fish that are too small, too young, or that appear diseased.

Systematic Sampling

Random sampling and judgmental sampling represent extremes in bias and in the number of samples needed to characterize the target population. ***Systematic sampling*** falls in between these extremes. In systematic sampling we sample the target population at regular intervals in space or time. Figure 7.2.1 shows an aerial photo of the Great Salt Lake in Utah. A railroad line divides the lake into two sections that have different chemical compositions. To compare the lake's two sections—and to evaluate spatial variations within each section—we use a two-dimensional grid to define sampling locations, collecting samples at the center of each location. When a population's is heterogeneous in time, as is common in clinical and environmental studies, then we might choose to collect samples at regular intervals in time.

What Type of Sample to Collect

Having determined from where to collect samples, the next step in designing a sampling plan is to decide on the type of sample to collect. There are three common methods for obtaining samples: grab sampling, composite sampling, and in situ sampling.

The most common type of sample is a ***grab sample*** in which we collect a portion of the target population at a specific time or location, providing a "snapshot" of the target population. If our target population is homogeneous, a series of random grab samples allows us to establish its properties. For a heterogeneous target population, systematic grab sampling allows us to characterize how its properties change over time and/or space.

A ***composite sample*** is a set of grab samples that we combine into a single sample before analysis. Because information is lost when we combine individual samples, normally we analyze separately each grab sample. In some situations, however, there are advantages to working with a composite sample.

One situation where composite sampling is appropriate is when our interest is in the target population's average composition over time or space. For example, wastewater treatment plants must monitor and report the average daily composition of the treated water they release to the environment. The analyst can collect and analyze a set of individual grab samples and report the average result, or she can save time and money by combining the grab samples into a single composite sample and report the result of her analysis of the composite sample.

Composite sampling also is useful when a single sample does not supply sufficient material for the analysis. For example, analytical methods for the quantitative analysis of PCB's in fish often require as much as 50 g of tissue, an amount that may be difficult to obtain from a single fish. Combining and homogenizing tissue samples from several fish makes it easy to obtain the necessary 50-g sample.

A significant disadvantage of grab samples and composite samples is that we cannot use them to monitor continuously a time-dependent change in the target population. ***In situ sampling***, in which we insert an analytical sensor into the target population, allows us to monitor the target population without removing individual grab samples. For example, we can monitor the pH of a solution in an industrial production line by immersing a pH electrode in the solution's flow.

✔ Example 7.2.2

A study of the relationship between traffic density and the concentrations of Pb, Cd, and Zn in roadside soils uses the following sampling plan [Nabulo, G.; Oryem-Origa, H.; Diamond, M. *Environ. Res.* **2006**, *101*, 42–52]. Samples of surface soil (0–10 cm) are collected at distances of 1, 5, 10, 20, and 30 m from the road. At each distance, 10 samples are taken from different locations and mixed to form a single sample. What type of sampling plan is this? Explain why this is an appropriate sampling plan.

Solution

This is a systematic–judgemental sampling plan using composite samples. These are good choices given the goals of the study. Automobile emissions release particulates that contain elevated concentrations of Pb, Cd, and Zn—this study was conducted in Uganda where leaded gasoline was still in use—which settle out on the surrounding roadside soils as "dry rain." Samples collected near the road and samples collected at fixed distances from the road provide sufficient data for the study, while minimizing the total number of samples. Combining samples from the same distance into a single, composite sample has the advantage of decreasing sampling uncertainty.

How Much Sample to Collect

To minimize sampling errors, samples must be of an appropriate size. If a sample is too small its composition may differ substantially from that of the target population, which introduces a sampling error. Samples that are too large, however, require more time and money to collect and analyze, without providing a significant improvement in the sampling error.

Let's assume our target population is a homogeneous mixture of two types of particles. Particles of type *A* contain a fixed concentration of analyte, and particles of type *B* are analyte-free. Samples from this target population follow a binomial distribution. If we collect a sample of *n* particles, then the expected number of particles that contains analyte, n_A, is

$$n_A = np$$

where *p* is the probability of selecting a particle of type *A*. The standard deviation for sampling is

$$s_{samp} = \sqrt{np(1-p)} \tag{7.2.1}$$

To calculate the relative standard deviation for sampling, $(s_{samp})_{rel}$, we divide Equation 7.2.1 by n_A, obtaining

$$(s_{samp})_{rel} = \frac{\sqrt{np(1-p)}}{np}$$

Solving for *n* allows us to calculate the number of particles we need to provide a desired relative sampling variance.

$$n = \frac{1-p}{p} \times \frac{1}{(s_{samp})^2_{rel}} \tag{7.2.2}$$

Example 7.2.3

Suppose we are analyzing a soil where the particles that contain analyte represent only 1×10^{-7}% of the population. How many particles must we collect to give a percent relative standard deviation for sampling of 1%?

Solution

Since the particles of interest account for 1×10^{-7}% of all particles, the probability, *p*, of selecting one of these particles is 1×10^{-9}. Substituting into Equation 7.2.2 gives

$$n = \frac{1-(1 \times 10^{-9})}{1 \times 10^{-9}} \times \frac{1}{(0.01)^2} = 1 \times 10^{13}$$

To obtain a relative standard deviation for sampling of 1%, we need to collect 1×10^{13} particles.

Depending on the particle size, a sample of 10^{13} particles may be fairly large. Suppose this is equivalent to a mass of 80 g. Working with a sample this large clearly is not practical. Does this mean we must work with a smaller sample and accept a larger relative standard deviation for sampling? Fortunately the answer is no. An important feature of Equation 7.2.2 is that the relative standard deviation for sampling is a function of the number of particles instead of their combined mass. If we crush and grind the particles to make them smaller, then a sample of 10^{13} particles will have a smaller mass. If we assume that a particle is spherical, then its mass is proportional to the cube of its radius.

$$\text{mass} \propto r^3$$

If we decrease a particle's radius by a factor of 2, for example, then we decrease its mass by a factor of 2^3, or 8. This assumes, of course, that the process of crushing and grinding particles does not change the composition of the particles.

Example 7.2.4

Assume that a sample of 10^{13} particles from Example 7.2.3 weighs 80 g and that the particles are spherical. By how much must we reduce a particle's radius if we wish to work with 0.6-g samples?

Solution

To reduce the sample's mass from 80 g to 0.6 g, we must change its mass by a factor of

$$\frac{80}{0.6} = 133\times$$

To accomplish this we must decrease a particle's radius by a factor of

$$r^3 = 133\times$$
$$r = 5.1\times$$

Decreasing the radius by a factor of approximately 5 allows us to decrease the sample's mass from 80 g to 0.6 g.

Treating a population as though it contains only two types of particles is a useful exercise because it shows us that we can improve the relative standard deviation for sampling by collecting more particles. Of course, a real population likely contains more than two types of particles, with the analyte present at several levels of concentration. Nevertheless, the sampling of many well-mixed populations approximate binomial sampling statistics because they are homogeneous on the scale at which they are sampled. Under these conditions the following relationship between the mass of a random grab sample, *m*, and the percent relative standard deviation for sampling, *R*, often is valid

$$mR^2 = K_s \tag{7.2.3}$$

where K_s is a sampling constant equal to the mass of a sample that produces a percent relative standard deviation for sampling of ±1% [Ingamells, C. O.; Switzer, P. *Talanta* **1973**, *20*, 547–568].

✔ Example 7.2.5

The following data were obtained in a preliminary determination of the amount of inorganic ash in a breakfast cereal.

mass of cereal (g)	0.9956	0.9981	1.0036	0.9994	1.0067
%w/w ash	1.34	1.29	1.32	1.26	1.28

What is the value of K_s and what size sample is needed to give a percent relative standard deviation for sampling of ±2.0%. Predict the percent relative standard deviation and the absolute standard deviation if we collect 5.00-g samples.

Solution

To determine the sampling constant, K_s, we need to know the average mass of the cereal samples and the relative standard deviation for the amount of ash in those samples. The average mass of the cereal samples is 1.0007 g. The average %w/w ash and its absolute standard deviation are, respectively, 1.298 %w/w and 0.03194 %w/w. The percent relative standard deviation, R, therefore, is

$$R = \frac{s_{\text{samp}}}{\overline{X}} = \frac{0.03194\%\ \text{w/w}}{1.298\%\ \text{w/w}} \times 100 = 2.46\%$$

Solving for K_s gives its value as

$$K_s = mR^2 = (1.0007\text{g})(2.46)^2 = 6.06\ \text{g}$$

To obtain a percent relative standard deviation of ±2%, samples must have a mass of at least

$$m = \frac{K_s}{R^2} = \frac{6.06\text{g}}{(2.0)^2} = 1.5\ \text{g}$$

If we use 5.00-g samples, then the expected percent relative standard deviation is

$$R = \sqrt{\frac{K_s}{m}} = \sqrt{\frac{6.06\text{g}}{5.00\text{g}}} = 1.10\%$$

and the expected absolute standard deviation is

$$s_{\text{samp}} = \frac{R\overline{X}}{100} = \frac{(1.10)(1.298\%\text{w/w})}{100} = 0.0143\%\text{w/w}$$

? Exercise 7.2.1

Olaquindox is a synthetic growth promoter in medicated feeds for pigs. In an analysis of a production lot of feed, five samples with nominal masses of 0.95 g were collected and analyzed, with the results shown in the following table.

mass (g)	0.9530	0.9728	0.9660	0.9402	0.9576
mg olaquindox/kg feed	23.0	23.8	21.0	26.5	21.4

What is the value of K_s and what size samples are needed to obtain a percent relative deviation for sampling of 5.0%? By how much do you need to reduce the average particle size if samples must weigh no more than 1 g?

Answer

How Many Samples to Collect

In the previous section we considered how much sample we need to minimize the standard deviation due to sampling. Another important consideration is the number of samples to collect. If the results from our analysis of the samples are normally distributed, then the confidence interval for the sampling error is

$$\mu = \overline{X} \pm \frac{ts_{samp}}{\sqrt{n_{samp}}} \tag{7.2.4}$$

where n_{samp} is the number of samples and s_{samp} is the standard deviation for sampling. Rearranging Equation 7.2.4 and substituting e for the quantity $\overline{X} - \mu$, gives the number of samples as

$$n_{samp} = \frac{t^2 s_{samp}^2}{e^2} \tag{7.2.5}$$

Because the value of t depends on n_{samp}, the solution to Equation 7.2.5 is found iteratively.

When we use Equation 7.2.5, we must express the standard deviation for sampling, s_{samp}, and the error, e, in the same way. If s_{samp} is reported as a percent relative standard deviation, then the error, e, is reported as a percent relative error. When you use Equation 7.2.5, be sure to check that you are expressing s_{samp} and e in the same way.

Example 7.2.6

In Example 7.2.5 we determined that we need 1.5-g samples to establish an s_{samp} of ±2.0% for the amount of inorganic ash in cereal. How many 1.5-g samples do we need to collect to obtain a percent relative sampling error of ±0.80% at the 95% confidence level?

Solution

Because the value of t depends on the number of samples—a result we have yet to calculate—we begin by letting $n_{samp} = \infty$ and using $t(0.05, \infty)$ for t. From Appendix 4, the value for $t(0.05, \infty)$ is 1.960. Substituting known values into Equation 7.2.5 gives the number of samples as

$$n_{samp} = \frac{(1.960)^2(2.0)^2}{(0.80)^2} = 24.0 \approx 24$$

Letting $n_{samp} = 24$, the value of $t(0.05, 23)$ from Appendix 4 is 2.073. Recalculating n_{samp} gives

$$n_{samp} = \frac{(2.073)^2(2.0)^2}{(0.80)^2} = 26.9 \approx 27$$

When $n_{samp} = 27$, the value of $t(0.05, 26)$ from Appendix 4 is 2.060. Recalculating n_{samp} gives

$$n_{samp} = \frac{(2.060)^2(2.0)^2}{(0.80)^2} = 26.52 \approx 27$$

Because two successive calculations give the same value for n_{samp}, we have an iterative solution to the problem. We need 27 samples to achieve a percent relative sampling error of ±0.80% at the 95% confidence level.

Minimizing the Overall Variance

A final consideration when we develop a sampling plan is how we can minimize the overall variance for the analysis. Equation 7.1.2 shows that the overall variance is a function of the variance due to the method, s^2_{meth}, and the variance due to sampling, s^2_{samp}. As we learned earlier, we can improve the sampling variance by collecting more samples of the proper size. Increasing the number of times we analyze each sample improves the method's variance. If s^2_{samp} is significantly greater than s^2_{meth}, we can ignore the method's contribution to the overall variance and use Equation 7.2.5 to estimate the number of samples to analyze. Analyzing any sample more than once will not improve the overall variance, because the method's variance is insignificant.

If s^2_{meth} is significantly greater than s^2_{samp}, then we need to collect and analyze only one sample. The number of replicate analyses, n_{rep}, we need to minimize the error due to the method is given by an equation similar to Equation 7.2.5.

$$n_{rep} = \frac{t^2 s^2_{meth}}{e^2}$$

Unfortunately, the simple situations described above often are the exception. For many analyses, both the sampling variance and the method variance are significant, and both multiple samples and replicate analyses of each sample are necessary. The overall error in this case is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp}n_{rep}}} \tag{7.2.6}$$

Equation 7.2.6 does not have a unique solution as different combinations of n_{samp} and n_{rep} give the same overall error. How many samples we collect and how many times we analyze each sample is determined by other concerns, such as the cost of collecting and analyzing samples, and the amount of available sample.

Example 7.2.7

An analytical method has a relative sampling variance of 0.40% and a relative method variance of 0.070%. Evaluate the percent relative error ($\alpha = 0.05$) if you collect 5 samples and analyze each twice, and if you collect 2 samples and analyze each 5 times.

Solution

Both sampling strategies require a total of 10 analyses. From Appendix 4 we find that the value of $t(0.05, 9)$ is 2.262. Using Equation 7.2.6, the relative error for the first sampling strategy is

$$e = 2.262\sqrt{\frac{0.40}{5} + \frac{0.070}{5 \times 2}} = 0.67\%$$

and that for the second sampling strategy is

$$e = 2.262\sqrt{\frac{0.40}{2} + \frac{0.070}{2 \times 5}} = 1.0\%$$

Because the method variance is smaller than the sampling variance, we obtain a smaller relative error if we collect more samples and analyze each sample fewer times.

Exercise 7.2.3

An analytical method has a relative sampling variance of 0.10% and a relative method variance of 0.20%. The cost of collecting a sample is \$20 and the cost of analyzing a sample is \$50. Propose a sampling strategy that provides a maximum relative error of ±0.50% ($\alpha = 0.05$) and a maximum cost of \$700.

Answer

If we collect a single sample (cost \$20), then we can analyze that sample 13 times (cost \$650) and stay within our budget. For this scenario, the percent relative error is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp}n_{rep}}} = 2.179\sqrt{\frac{0.10}{1} + \frac{0.20}{1 \times 13}} = 0.74\%$$

where $t(0.05, 12)$ is 2.179. Because this percent relative error is larger than ±0.50%, this is not a suitable sampling strategy.

Next, we try two samples (cost \$40), analyzing each six times (cost \$600). For this scenario, the percent relative error is

$$e = t\sqrt{\frac{s^2_{samp}}{n_{samp}} + \frac{s^2_{meth}}{n_{samp}n_{rep}}} = 2.2035\sqrt{\frac{0.10}{2} + \frac{0.20}{2 \times 6}} = 0.57\%$$

where $t(0.05, 11)$ is 2.2035. Because this percent relative error is larger than ±0.50%, this also is not a suitable sampling strategy.

Next we try three samples (cost \$60), analyzing each four times (cost \$600). For this scenario, the percent relative error is

$$e = t\sqrt{\frac{s_{samp}^2}{n_{samp}} + \frac{s_{meth}^2}{n_{samp}n_{rep}}} = 2.2035\sqrt{\frac{0.10}{3} + \frac{0.20}{3 \times 4}} = 0.49\%$$

where $t(0.05, 11)$ is 2.2035. Because both the total cost (\$660) and the percent relative error meet our requirements, this is a suitable sampling strategy.

There are other suitable sampling strategies that meet both goals. The strategy that requires the least expense is to collect eight samples, analyzing each once for a total cost of \$560 and a percent relative error of ±0.46%. Collecting 10 samples and analyzing each one time, gives a percent relative error of ±0.39% at a cost of \$700.

7.3: Implementing the Sampling Plan

Implementing a sampling plan usually involves three steps: physically removing the sample from its target population, preserving the sample, and preparing the sample for analysis. Except for in situ sampling, we analyze a sample after we have removed it from its target population. Because sampling exposes the target population to potential contamination, our sampling device must be inert and clean.

Once we remove a sample from its target population, there is a danger that it will undergo a chemical or physical change before we can complete its analysis. This is a serious problem because the sample's properties will no longer e representative of the target population. To prevent this problem, we often preserve samples before we transport them to the laboratory for analysis. Even when we analyze a sample in the field, preservation may still be necessary.

The initial sample is called the primary or ***gross sample***, and it may be a single increment drawn from the target population or a composite of several increments. In many cases we cannot analyze the gross sample without first preparing the sample for analyze by reducing the sample's particle size, by converting the sample into a more readily analyzable form, or by improving its homogeneity.

Although you may never work with the specific samples highlighted in this section, the case studies presented here may help you in envisioning potential problems associated with your samples.

Sample Collection

The chemical composition of a surface water—such as a stream, river, lake, estuary, or ocean—is influenced by flow rate and depth. Rapidly flowing shallow streams and rivers, and shallow (<5 m) lakes usually are well mixed and show little stratification with depth. To collect a grab sample we submerge a capped bottle below the surface, remove the cap and allow the bottle to fill completely, and replace the cap. Collecting a sample this way avoids the air–water interface, which may be enriched with heavy metals or contaminated with oil [Duce, R. A.; Quinn, J. G. Olney, C. E.; Piotrowicz, S. R.; Ray, S. J.; Wade, T. L. *Science* **1972**, *176*, 161–163].

Slowly moving streams and rivers, lakes deeper than five meters, estuaries, and oceans may show substantial stratification with depth. Grab samples from near the surface are collected as described above, and samples at greater depths are collected using a sample bottle lowered to the desired depth (Figure 7.3.1).

Figure 7.3.1 . A Niskin sampling bottle for collecting water samples from lakes and oceans. After lowering the bottle to the desired depth, a weight is sent down a winch line, tripping a spring that closes the bottle. Source: modified from NOAA.

Wells for sampling groundwater are purged before we collect samples because the chemical composition of water in a well-casing may differ significantly from that of the groundwater. These differences may result from contaminants introduced while drilling the well or by a change in the groundwater's redox potential following its exposure to atmospheric oxygen. In general, a well is purged by pumping out a volume of water equivalent to several well-casing volumes or by pumping until the water's temperature, pH, or specific conductance is constant. A municipal water supply, such as a residence or a business, is purged before sampling because the chemical composition of water standing in a pipe may differ significantly from the treated water supply. Samples are collected at faucets after flushing the pipes for 2-3 minutes.

Samples from municipal wastewater treatment plants and industrial discharges often are collected as a 24-hour composite. An automatic sampler periodically removes an individual grab sample, adding it to those collected previously. The volume of each sample and the frequency of sampling may be constant, or may vary in response to changes in flow rate.

Sample containers for collecting natural waters and wastewaters are made from glass or plastic. Kimax and Pyrex brand borosilicate glass have the advantage of being easy to sterilize, easy to clean, and inert to all solutions except those that are strongly alkaline. The disadvantages of glass containers are cost, weight, and the ease of breakage. Plastic containers are made from a variety of polymers, including polyethylene, polypropylene, polycarbonate, polyvinyl chloride, and Teflon. Plastic containers are light-weight, durable, and, except for those manufactured from Teflon, inexpensive. In most cases glass or plastic bottles are used interchangeably, although polyethylene bottles generally are preferred because of their lower cost. Glass containers are always used when collecting samples for the analysis of pesticides, oil and grease, and organics because these species often interact with plastic surfaces. Because glass surfaces easily adsorb metal ions, plastic bottles are preferred when collecting samples for the analysis of trace metals.

In most cases the sample bottle has a wide mouth, which makes it easy to fill and to remove the sample. A narrow-mouth sample bottle is used if exposing the sample to the container's cap or to the outside environment is a problem. Unless exposure to plastic is a problem, caps for sample bottles are manufactured from polyethylene. When polyethylene must be avoided, the container's cap includes an inert interior liner of neoprene or Teflon.

Sample Preservation and Preparation

Here our concern is only with the need to prepare the gross sample by converting it into a form suitable for analysis. Some analytical methods require additional sample preparation steps, such as concentrating or diluting the analyte, or adjusting the analyte's chemical form. We will consider these forms of sample preparation in later chapters that focus on specific analytical methods.

After removing a sample from its target population, its chemical composition may change as a result of chemical, biological, or physical processes. To prevent a change in composition, samples are preserved by controlling the sample's pH and temperature, by limiting its exposure to light or to the atmosphere, or by adding a chemical preservative. After preserving a sample, it is safely stored for later analysis. The maximum holding time between preservation and analysis depends on the analyte's stability and the effectiveness of sample preservation. Table 7.3.1 summarizes preservation methods and maximum holding times for several analytes of importance in the analysis of natural waters and wastewaters.

Table 7.3.1 . Preservation Methods and Maximum Holding Times for Selected Analytes in Natural Waters and Wastewaters

analyte	preservation method	maximum holding time
ammonia	cool to 4°C; add H_2SO_4 to pH < 2	28 days
chloride	none required	28 days
metals: Cr(VI)	cool to 4°C	24 hours
metals: Hg	HNO_3 to pH < 2	28 days
metals: all others	HNO_3 to pH < 2	6 months
nitrate	none required	48 hours
organochlorine pesticides	1 mL of 10 mg/mL $HgCl_2$ or immediate extraction with a suitable non-aqueous solvent	7 days without extraction; 40 days with extraction
pH	none required	analyze immediately

Other than adding a preservative, solution samples generally do not need additional preparation before analysis. This is the case for samples of natural waters and wastewaters. Solution samples with particularly complex matricies—blood and milk are two common examples—may need addi- tional processing to separate analytes from interferents, a topic covered later in this chapter.

Gases

Typical examples of gaseous samples include automobile exhaust, emissions from industrial smokestacks, atmospheric gases, and compressed gases. Also included in this category are aerosol particulates—the fine solid particles and liquid droplets that form smoke and smog. Let's use the sampling of urban air as a case study in how to sample a gas.

Sample Collection

One approach for collecting a sample of urban air is to fill a stainless steel canister or a Tedlar/Teflon bag. A pump pulls the air into the container and, after purging, the container is sealed. This method has the advantage of being simple and of collecting a representative sample. Disadvantages include the tendency for some analytes to adsorb to the container's walls, the presence of analytes at concentrations too low to detect with suitable accuracy and precision, and the presence of reactive analytes, such as ozone and nitrogen oxides, that may react with the container or that may otherwise alter the sample's chemical composition during storage. When using a stainless steel canister, cryogenic cooling, which changes the sample from a gaseous state to a liquid state, may limit some of these disadvantages.

Most urban air samples are collected by filtration or by using a trap that contains a solid sorbent. Solid sorbents are used for volatile gases (a vapor pressure more than 10^{-6} atm) and for semi-volatile gases (a vapor pressure between 10^{-6} atm and 10^{-12} atm). Filtration is used to collect aerosol particulates. Trapping and filtering allow for sampling larger volumes of gas—an important concern for an analyte with a small concentration—and stabilizes the sample between its collection and its analysis.

In solid sorbent sampling, a pump pulls the urban air through a canister packed with sorbent particles. Typically 2–100 L of air are sampled when collecting a volatile compound and 2–500 m^3 when collecting a semi-volatile gas. A variety of inorganic, organic polymer, and carbon sorbents have been used. Inorganic sorbents, such as silica gel, alumina, magnesium aluminum silicate, and molecular sieves, are efficient collectors for polar compounds. Their efficiency at absorbing water, however, limits their capacity for many organic analytes.

> 1 m^3 is equivalent to 10^3 L.

Organic polymeric sorbents include polymeric resins of 2,4-diphenyl-*p*-phenylene oxide or styrene-divinylbenzene for volatile compounds, and polyurethane foam for semi-volatile compounds. These materials have a low affinity for water and are efficient for sampling all but the most highly volatile organic compounds and some lower molecular weight alcohols and ketones. Carbon sorbents are superior to organic polymer resins, which makes them useful for highly volatile organic compounds that will not absorb onto polymeric resins, although removing the compounds may be difficult.

Non-volatile compounds normally are present either as solid particulates or are bound to solid particulates. Samples are collected by pulling a large volume of urban air through a filtering unit and collecting the particulates on glass fiber filters.

The short term exposure of humans, animals, and plants to atmospheric pollutants is more severe than that for pollutants in other matrices. Because the composition of atmospheric gases can vary significantly over a time, the continuous monitoring of atmospheric gases such as O_3, CO, SO_2, NH_3, H_2O_2, and NO_2 by in situ sampling is important [Tanner, R. L. in Keith, L. H., ed. *Principles of Environmental Sampling*, American Chemical Society: Washington, D. C., 1988, 275–286].

Sample Preservation

After collecting a gross sample of urban air, generally there is little need for sample preservation or preparation. The chemical composition of a gas sample usually is stable when it is collected using a solid sorbent, a filter, or by cryogenic cooling. When using a solid sorbent, gaseous compounds are released for analysis by thermal desorption or by extracting with a suitable solvent. If the sorbent is selective for a single analyte, the increase in the sorbent's mass is used to determine the amount of analyte in the sample.

Solids

Typical examples of solid samples include large particulates, such as those found in ores; smaller particulates, such as soils and sediments; tablets, pellets, and capsules used for dispensing pharmaceutical products and animal feeds; sheet materials, such as polymers and rolled metals; and tissue samples from biological specimens. Solids usually are heterogeneous and we must collect samples carefully if they are to be representative of the target population. Let's use the sampling of sediments, soils, and ores as a case study in how to sample solids.

Sample Collection

Sediments from the bottom of streams, rivers, lakes, estuaries, and oceans are collected with a bottom grab sampler or with a corer. A bottom grab sampler (Figure 7.3.2) is equipped with a pair of jaws that close when they contact the sediment, scooping up sediment in the process. Its principal advantages are ease of use and the ability to collect a large sample. Disadvantages include the tendency to lose finer grain sediment particles as water flows out of the sampler, and the loss of spatial information—both laterally and with depth—due to mixing of the sample.

Figure 7.3.2 . Bottom grab sampler being prepared for deployment. Source: NOAA.

An alternative method for collecting sediments is the cylindrical coring device shown in Figure 7.3.3). The corer is dropped into the sediment, collecting a column of sediment and the water in contact with the sediment. With the possible exception of sediment at the surface, which may experience mixing, samples collected with a corer maintain their vertical profile, which preserves information about how the sediment's composition changes with depth.

Collecting soil samples at depths of up to 30 cm is accomplished with a scoop or a shovel, although the sampling variance generally is high. A better tool for collecting soil samples near the surface is a soil punch, which is a thin-walled steel tube that retains a core sample after it is pushed into the soil and removed. Soil samples from depths greater than 30 cm are collected by digging a trench and collecting lateral samples with a soil punch. Alternatively, an auger is used to drill a hole to the desired depth and the sample collected with a soil punch.

For particulate materials, particle size often determines the sampling method. Larger particulate solids, such as ores, are sampled using a riffle (Figure 7.3.4), which is a trough with an even number of compartments. Because adjoining compartments empty onto opposite sides of the riffle, dumping a gross sample into the riffle divides it in half. By repeatedly passing half of the separated material back through the riffle, a sample of the desired size is collected.

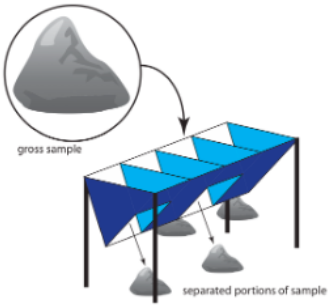


Figure 7.3.4 . Example of a four-unit riffle. Passing the gross sample, shown within the circle, through the riffle divides it into four piles, two on each side. Combining the piles from one side of the riffle provides a new sample, which is passed through the riffle again or kept as the final sample. The piles from the other side of the riffle are discarded.

A sample thief (Figure 7.3.5) is used for sampling smaller particulate materials, such as powders. A typical sample thief consists of two tubes that are nestled together. Each tube has one or more slots aligned down the length of the sample thief. Before inserting the sample thief into the material being sampled, the slots are closed by rotating the inner tube. When the sample thief is in place, rotating the inner tube opens the slots, which fill with individual samples. The inner tube is then rotated to the closed position and the sample thief withdrawn.

Sample Preservation

Without preservation, a solid sample may undergo a change in composition due to the loss of volatile material, biodegradation, or chemical reactivity (particularly redox reactions). Storing samples at lower temperatures makes them less prone to biodegradation and to the loss of volatile material, but fracturing of solids and phase separations may present problems. To minimize the loss of volatile compounds, the sample container is filled completely, eliminating a headspace where gases collect. Samples that have not been exposed to O_2 particularly are susceptible to oxidation reactions. For example, samples of anaerobic sediments must be prevented from coming into contact with air.

Sample Preparation

Unlike gases and liquids, which generally require little sample preparation, a solid sample usually needs some processing before analysis. There are two reasons for this. First, as discussed in Chapter 7.2, the standard deviation for sampling, s_{samp}, is a function of the number of particles in the sample, not the combined mass of the particles. For a heterogeneous material that consists of large particulates, the gross sample may be too large to analyze. For example, a Ni-bearing ore with an average particle size of 5 mm may require a sample that weighs one ton to obtain a reasonable s_{samp}. Reducing the sample's average particle size allows us to collect the same number of particles with a smaller, more manageable mass. Second, many analytical techniques require that the analyte be in solution.

Reducing Particle Size

A reduction in particle size is accomplished by crushing and grinding the gross sample. The resulting particulates are then thoroughly mixed and divided into ***subsamples*** of smaller mass. This process seldom occurs in a single step. Instead, subsamples are cycled through the process several times until a final ***laboratory sample*** is obtained.

Crushing and grinding uses mechanical force to break larger particles into smaller particles. A variety of tools are used depending on the particle's size and hardness. Large particles are crushed using jaw crushers that can reduce particles to diameters of a few millimeters. Ball mills, disk mills, and mortars and pestles are used to further reduce particle size.

A significant change in the gross sample's composition may occur during crushing and grinding. Decreasing particle size increases the available surface area, which increases the risk of losing volatile components. This problem is made worse by the frictional heat that accompanies crushing and grinding. Increasing the surface area also exposes interior portions of the sample to the atmosphere where oxidation may alter the gross sample's composition. Other problems include contamination from the materials used to crush and grind the sample, and differences in the ease with which particles are reduced in size. For example, softer particles are easier to reduce in size and may be lost as dust before the remaining sample is processed. This is a particular problem if the analyte's distribution between different types of particles is not uniform.

The gross sample is reduced to a uniform particle size by intermittently passing it through a sieve. Those particles not passing through the sieve receive additional processing until the entire sample is of uniform size. The resulting material is mixed thoroughly to ensure homogeneity and a subsample obtained with a riffle, or by c***oning and quartering***. As shown in Figure 7.3.6 , the gross sample is piled into a cone, flattened, and divided into four quarters. After discarding two diagonally opposed quarters, the remaining material is cycled through the process of coning and quartering until a suitable laboratory sample remains.

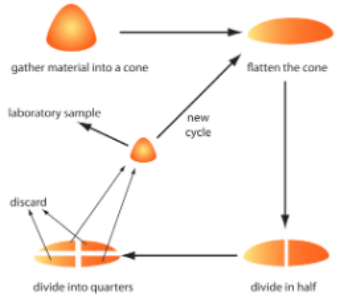


Figure 7.3.6 . Illustration showing the method of coning and quartering for reducing sample size. After gathering the gross sample into a cone, the cone is flattened, divided in half, and then divided into quarters. Two opposing quarters are combined to form the laboratory sample or a subsample that is sent through another cycle. The two remaining quarters are discarded.

Bringing Solid Samples Into Solution

If you are fortunate, your sample will dissolve easily in a suitable solvent, requiring no more effort than gently swirling and heating. Distilled water usually is the solvent of choice for inorganic salts, but organic solvents, such as methanol, chloroform, and toluene, are useful for organic materials.

When a sample is difficult to dissolve, the next step is to try digesting it with an acid or a base. Table 7.3.2 lists several common acids and bases, and summarizes their use. Digestions are carried out in an open container, usually a beaker, using a hot-plate as a source of heat. The main advantage of an open-vessel digestion is cost because it requires no special equipment. Volatile reaction products, however, are lost, which results in a determinate error if they include the analyte.

Table 7.3.2 . Acids and Bases Used for Digesting Samples

solution	uses and properties
HCl (37% w/w)	• dissolves metals more easily reduced than H_2 ($E^o < 0$) • dissolves insoluble carbonate, sulfides, phosphates, fluorides, sulfates, and many oxides
HNO_3 (70% w/w)	• strong oxidizing agent • dissolves most common metals except Al, Au, Pt, and Cr • decomposes organics and biological samples (wet ashing)
H_2SO_4 (98% w/w)	• dissolves many metals and alloys • decomposes organics by oxidation and dehydration
HF (50% w/w)	• dissolves silicates by forming volatile SiF_4
$HClO_4$ (70% w/w)	• hot, concentrated solutions are strong oxidizing agents • dissolves many metals and alloys • decomposes organics (*Caution: reactions with organics often are explosive; use only in a specially equipped hood with a blast shield and after prior decomposition with HNO_3*)
$HCl:HNO_3$ (3:1 v/v)	• also known as aqua regia • dissolves Au and Pt
NaOH	• dissolves Al and amphoteric oxides of Sn, Pb, Zn, and Cr

7.4: Separating the Analyte From Interferents

When an analytical method is selective for the analyte, analyzing a sample is a relatively simple task. For example, a quantitative analysis for glucose in honey is relatively easy to accomplish if the method is selective for glucose, even in the presence of other reducing sugars, such as fructose. Unfortunately, few analytical methods are selective toward a single species.

In the absence of an interferent, the relationship between the sample's signal, S_{samp}, and the analyte's concentration, C_A, is

$$S_{samp} = k_A C_A \tag{7.4.1}$$

where k_A is the analyte's sensitivity.

> In Equation 7.4.1, and the equations that follow, you can replace the analyte's concentration, C_A, with the moles of analyte, n_A, when working with methods, such as gravimetry, that respond to the absolute amount of analyte in a sample. In this case the interferent also is expressed in terms of moles.

If an interferent, is present, then Equation 7.4.1 becomes

$$S_{samp} = k_A C_A + k_I C_I \tag{7.4.2}$$

where k_I and C_I are, respectively, the interferent's sensitivity and concentration. A method's selectivity for the analyte is determined by the relative difference in its sensitivity toward the analyte and the interferent. If k_A is greater than k_I, then the method is more selective for the analyte. The method is more selective for the interferent if k_I is greater than k_A.

Even if a method is more selective for an interferent, we can use it to determine C_A if the interferent's contribution to S_{samp} is insignificant. The ***selectivity coefficient***, $K_{A,I}$, which we introduced in Chapter 3, provides a way to characterize a method's selectivity.

$$K_{A,I} = \frac{k_I}{k_A} \tag{7.4.3}$$

Solving Equation 7.4.3 for k_I, substituting into Equation 7.4.2, and simplifying, gives

$$S_{samp} = k_A \left(C_A + K_{A,I} \times C_I\right) \tag{7.4.4}$$

An interferent, therefore, does not pose a problem as long as the product of its concentration and its selectivity coefficient is significantly smaller than the analyte's concentration.

$$K_{A,I} \times C_I << C_A$$

If we cannot ignore an interferent's contribution to the signal, then we must begin our analysis by separating the analyte and the interferent.

7.5: General Theory of Separation Effiiciency

The goal of an analytical separation is to remove either the analyte or the interferent from the sample's matrix. To achieve this separation we must identify at least one significant difference between the analyte's and the interferent's chemical or physical properties. A significant difference in properties, however, is not sufficient to effect a separation if the conditions that favor the extraction of interferent from the sample also removes a small amount of analyte.

Two factors limit a separation's efficiency: failing to recover all the analyte and failing to remove all the interferent. We define the analyte's ***recovery***, R_A, as

$$R_A = \frac{C_A}{(C_A)_o} \tag{7.5.1}$$

where C_A is the concentration of analyte that remains after the separation, and $(C_A)_o$ is the analyte's initial concentration. A recovery of 1.00 means that no analyte is lost during the separation. The interferent's recovery, R_I, is defined in the same manner

$$R_I = \frac{C_I}{(C_I)_o} \tag{7.5.2}$$

where C_I is the concentration of interferent that remains after the separation, and $(C_I)_o$ is the interferent's initial concentration. We define the extent of the separation using a ***separation factor***, $S_{I,A}$ [(a) Sandell, E. B. *Colorimetric Determination of Trace Metals*, Interscience Publishers: New York, 1950, pp. 19–20; (b) Sandell, E. B. *Anal. Chem.* **1968**, *40*, 834–835].

$$S_{I,A} = \frac{R_I}{R_A} \tag{7.5.3}$$

In general, an $S_{I,A}$ of approximately 10^{-7} is needed for the quantitative analysis of a trace analyte in the presence of a macro interferent, and 10^{-3} when the analyte and interferent are present in approximately equal amounts.

> The meaning of trace and macro, as well as other terms for describing the concentrations of analytes and interferents, is presented in Chapter 2.

✔ Example 7.5.1

An analytical method for determining Cu in an industrial plating bath gives poor results in the presence of Zn. To evaluate a method for separating the analyte from the interferent, samples with known concentrations of Cu or Zn were prepared and analyzed. When a sample of 128.6 ppm Cu was taken through the separation, the concentration of Cu that remained was 127.2 ppm. Taking a 134.9 ppm solution of Zn through the separation left behind a concentration of 4.3 ppm Zn. Calculate the recoveries for Cu and Zn, and the separation factor.

Solution

Using Equation 7.5.1 and Equation 7.5.2, the recoveries for the analyte and interferent are

$$R_{Cu} = \frac{127.2 \text{ ppm}}{128.6 \text{ ppm}} = 0.9891 \text{ or } 98.91\%$$

$$R_{zn} = \frac{4.3 \text{ ppm}}{134.9 \text{ ppm}} = 0.032 \text{ or } 3.2\%$$

and the separation factor is

$$S_{Zn,Cu} = \frac{R_{Zn}}{R_{Cu}} = \frac{0.032}{0.9891} = 0.032$$

Recoveries and separation factors are useful tools for evaluating a separation's potential effectiveness; they do not, however, give a direct indication of the error that results from failing to remove all the interferent or from failing to completely recover the analyte. The relative error due to the separation, E, is

$$E = \frac{S_{samp} - S^*_{samp}}{S_{samp}} \tag{7.5.4}$$

where S^*_{samp} is the sample's signal for an ideal separation in which we completely recover the analyte.

$$S^*_{samp} = k_A(C_A)_o \tag{7.5.5}$$

Substituting equation 7.4.4 and Equation 7.5.5 into Equation 7.5.4, and rearranging

$$E = \frac{k_A(C_A + K_{A,I} \times C_I) - k_A(C_A)_o}{k_A(C_A)_o}$$

$$E = \frac{C_A + K_{A,I} \times C_I - (C_A)_o}{(C_A)_o}$$

$$E = \frac{C_A}{(C_A)_o} - \frac{(C_A)_o}{(C_A)_o} + \frac{K_{A,I} \times C_I}{(C_A)_o}$$

leaves us with

$$E = (R_A - 1) + \frac{K_{A,I} \times C_I}{(C_A)_o} \tag{7.5.6}$$

A more useful equation is obtained by solving Equation 7.5.2 for C_I and substituting into Equation 7.5.6.

$$E = (R_A - 1) + \frac{K_{A,I} \times (C_I)_o}{(C_A)_o} \times R_I \tag{7.5.7}$$

The first term of Equation 7.5.7 accounts for the analyte's incomplete recovery and the second term accounts for a failure to remove all the interferent.

✔ Example 7.5.2

Following the separation outlined in Example 7.5.1 , an analysis is carried out to determine the concentration of Cu in an industrial plating bath. Analysis of standard solutions that contain either Cu or Zn give the following linear calibrations.

$$S_{Cu} = 1250\ \text{ppm}^{-1} \times C_{Cu} \text{ and } S_{Zn} = 2310\ \text{ppm}^{-1} \times C_{Zn}$$

(a) What is the relative error if we analyze a sample without removing the Zn? Assume the initial concentration ratio, Cu:Zn, is 7:1. (b) What is the relative error if we first complete the separation with the recoveries determined in Example 7.5.1 ? (c) What is the maximum acceptable recovery for Zn if the recovery for Cu is 1.00 and if the error due to the separation must be no greater than 0.10%?

Solution

(a) If we complete the analysis without separating Cu and Zn, then R_{Cu} and R_{Zn} are exactly 1 and Equation 7.5.7 simplifies to

$$E = \frac{K_{Cu,Zn} \times (C_{Zn})_o}{(C_{Cu})_o}$$

Using equation 7.4.3, we find that the selectivity coefficient is

$$K_{Cu,Zn} = \frac{k_{Zn}}{k_{Cu}} = \frac{2310\ \text{ppm}^{-1}}{1250\ \text{ppm}^{-1}} = 1.85$$

Given the initial concentration ratio of 7:1 for Cu and Zn, the relative error without the separation is

$$E = \frac{1.85 \times 1}{7} = 0.264 \text{ or } 26.4\%$$

(b) To calculate the relative error we substitute the recoveries from Example 7.5.1 into Equation 7.5.7, obtaining

$$E = (0.9891 - 1) + \frac{1.85 \times 1}{7} \times 0.032 = -0.0109 + 0.085 = -0.0024$$

or –0.24%. Note that the negative determinate error from failing to recover all the analyte is offset partially by the positive determinate error from failing to remove all the interferent.

(c) To determine the maximum recovery for Zn, we make appropriate substitutions into Equation 7.5.7

$$E = 0.0010 = (1 - 1) + \frac{1.85 \times 1}{7} \times R_{Zn}$$

and solve for R_{Zn}, obtaining a recovery of 0.0038, or 0.38%. Thus, we must remove at least

$$100.00\% - 0.38\% = 99.62\%$$

of the Zn to obtain an error of 0.10% when R_{Cu} is exactly 1.

7.6: Classifying Separation Techniques

We can separate an analyte and an interferent if there is a significant difference in at least one of their chemical or physical properties. Table 7.6.1 provides a partial list of separation techniques, organized by the chemical or physical property affecting the separation.

Table 7.6.1 . Classification of Separation Techniques

basis of separation	separation technique(s)
size	filtration; dialaysis; size-exclusion chromatography
mass or density	centrifugation
complex formation	masking
change in physical state	distillation; sublimation; recrystalization
change in chemical state	precipitation; electrodeposition; volatilization
partitioning between phases	extraction; chromatography

Separations Based on Size

Size is the simplest physical property we can exploit in a separation. To accomplish the separation we use a porous medium through which only the analyte or the interferent can pass. Examples of size-based separations include filtration, dialysis, and size-exclusion.

In a ***filtration*** we separate a particulate interferent from soluble analytes using a filter with a pore size that will retain the interferent. The solution that passes through the filter is called the ***filtrate***, and the material retained by the filter is the ***retentate***. Gravity filtration and suction filtration using filter paper are techniques with which you should already be familiar. A membrane filter is the method of choice for particulates that are too small to be retained by filter paper. Figure 7.6.1 provides information about three types of membrane filters. For applications of gravity filtration and suction filtration in gravimetric methods of analysis, see Chapter 8.

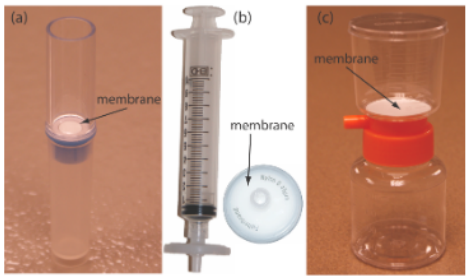


Figure 7.6.1 . Examples of three types of membrane filters for separating analytes and interferents. (a) A centrifugal filter for concentrating and desalting macromolecular solutions. The membrane has a nominal molecular weight cut-off of 1×10^6 g/mol. The sample is placed in the upper reservoir and the unit is placed in a centrifuge. Upon spinning the unit at $2000 \times g - 5000 \times g$, the filtrate collects in the bottom reservoir and the retentate remains in the upper reservoir. (b) A 0.45 µm membrane syringe filter. The photo on the right shows the membrane filter in its casing. In the photo on the left, the filter is attached to a syringe. Samples are placed in the syringe and pushed through the filter. The filtrate is collected in a test tube or other suitable container. (c) A disposable filter system with a 0.22 µm cellulose acetate membrane filter. The sample is added to the upper unit and vacuum suction is used to draw the filtrate through the membrane and into the lower unit. To store the filtrate, the top half of the unit is removed and a cap placed on the lower unit. The filter unit shown here has a capacity of 150 mL.

Dialysis is another example of a separation technique in which size is used to separate the analyte and the interferent. A dialysis membrane usually is made using cellulose and fashioned into tubing, bags, or cassettes. Figure 7.6.2 shows an example of a commercially available dialysis cassette. The sample is injected into the dialysis membrane, which is sealed tightly by a gasket, and the unit is placed in a container filled with a solution with a composition different from the sample. If there is a difference in a species' concentration on the membrane's two sides, the resulting concentration gradient provides a driving force for its diffusion across the membrane. While small species freely pass through the membrane, larger species are unable to pass. Dialysis frequently is used to purify proteins, hormones, and enzymes. During kidney dialysis, metabolic waste products, such as urea, uric acid, and creatinine, are removed from blood by passing it over a dialysis membrane.

Size-exclusion chromatography is a third example of a separation technique that uses size as a means to effect a separation. In this technique a column is packed with small, approximately 10-µm, porous polymer beads of cross-linked dextrin or polyacrylamide. The pore size of the particles is controlled by the degree of cross-linking, with more cross-linking producing smaller pore sizes. The sample is placed into a stream of solvent that is pumped through the column at a fixed flow rate. Those species too large to enter the pores pass through the column at the same rate as the solvent. Species that enter into the pores take longer to pass through the column, with smaller species requiring more time to pass through the column. Size-exclusion chromatography is widely used in the analysis of polymers, and in biochemistry, where it is used for the separation of proteins. A more detailed treatment of size-exclusion chromatography, which also is called gel permeation chromatography, is in Chapter 12.

Separations Based on Mass or Density

If the analyte and the interferent have different masses or densities, then a separation using ***centrifugation*** may be possible. The sample is placed in a centrifuge tube and spun at a high angular velocity, measured in revolutions per minute (rpm). The sample's constituents experience a centrifugal force that pulls them toward the bottom of the centrifuge tube. Those species that experience the greatest centrifugal force have the fastest sedimentation rate and are the first to reach the bottom of the centrifuge tube. If two species have the same density, their separation is based on a difference in mass, with the heavier species having the greater sedimentation rate. If the species are of equal mass, then the species with the larger density has the greatest sedimentation rate.

Centrifugation is an important separation technique in biochemistry. Table 7.6.2 , for example, lists conditions for separating selected cellular components. We can separate lysosomes from other cellular components by several differential centrifugations, in which we divide the sample into a solid residue and a supernatant solution. After destroying the cells, the solution is centrifuged for 20 minutes at $15000 \times g$ (a centrifugal force that is 15 000 times the earth's gravitational force), leaving a solid residue of cell membranes and mitochondria. The supernatant, which contains the lysosomes, is isolated by decanting it from the residue and then centrifuged for 30 minutes at $30000 \times g$, leaving a solid residue of lysosomes. Figure 7.6.3 shows a typical centrifuge capable of producing the centrifugal forces needed for biochemical separations.

Table 7.6.2 . Conditions for Separating Selected Cellular Components by Centrifugation

components	centrifugal force ($\times g$)	time (min)
eukaryotic cells	1000	5
cell membranes; nuclei	4000	10
mitochondria, bacterial cells	15000	20
lysosomes; bacterial membranes	30000	30
ribosomes	100000	180

Source: Adapted from Zubay, G. *Biochemistry*, 2nd ed. Macmillan: New York, 1988, p.120.

Figure 7.6.3 . Bench-top centrifuge capable of reaching speeds up to 14000 rpm and centrifugal forces of 20800 $\times$ g. This particular centrifuge is refrigerated, allowing samples to be cooled to temperatures as low as –4°C.

An alternative approach to differential centrifugation is a density gradient centrifugation. To prepare a sucrose density gradient, for example, a solution with a smaller concentration of sucrose—and, thus, of lower density—is gently layered upon a solution with a higher concentration of sucrose. Repeating this process several times, fills the centrifuge tube with a multi-layer density gradient. The sample is placed on top of the density gradient and centrifuged using a force greater than 150000 $\times$ g. During centrifugation, each of the sample's components moves through the gradient until it reaches a position where its density matches the surrounding sucrose solution. Each component is isolated as a separate band positioned where its density is equal to that of the local density within the gradient. Figure 7.6.4 provides an example of a typical sucrose density centrifugation for separating plant thylakoid membranes.

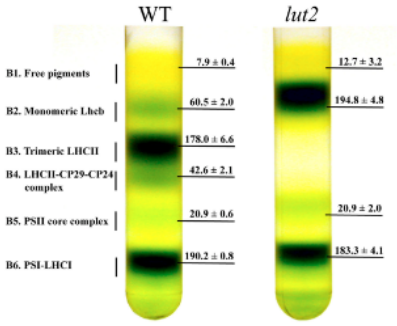


Figure 7.6.4 . Example of a sucrose density gradient centrifugation of thylakoid membranes from wild type (WT) and *lut2* plants. The thylakoid membranes were extracted from the plant's leaves and separated by centrifuging in a 0.1–1 M sucrose gradient for 22 h at 280000 $\times$ g and at 4°C. Six bands and their chlorophyll contents are shown. Adapted from Dall'Osto, L.; Lico, C.; Alric, J.; Giuliano, G.; Havaux, M.; Bassi, R. *BMC Plant Biology* **2006**, *6:32*.

Separations Based on Complexation Reactions (Masking)

One widely used technique for preventing an interference is to bind the interferent in a strong, soluble complex that prevents it from interfering in the analyte's determination. This process is known as ***masking***. As shown in Table 7.6.3 , a wide variety of ions and molecules are useful ***masking agents***, and, as a result, selectivity is usually not a problem.

> Technically, masking is not a separation technique because we do not physically separate the analyte and the interferent. We do, however, chemically isolate the interferent from the analyte, resulting in a pseudo-separation.

Table 7.6.3 . Selected Inorganic and Organic Masking Agents for Metal Ions

masking agent	elements whose ions are masked
CN^-	Ag, Au, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pd, Pt, Zn
SCN^-	Ag, Cd, Co, Cu, Fe, Ni, Pd, Pt, Zn
NH_3	Ag, Co, Ni, Cu, Zn
F^-	Al, Co, Cr, Mg, Mn, Sn, Zn

$S_2O_3^{2-}$	Au, Ce, Co, Cu, Fe, Hg, Mn, Pb, Pd, Pt, Sb, Sn, Zn
tartrate	Al, Ba, Bi, Ca, Ce, Co, Cr, Cu, Fe, Hg, Mn, Pb, Pd, Pt, Sb, Sn, Zn
oxalate	Al, Fe, Mg, Mn
thioglycolic acid	Cu, Fe, Sn

Source: Meites, L. *Handbook of Analytical Chemistry*, McGraw-Hill: New York, 1963.

✔ Example 7.6.1

Using Table 7.6.3 , suggest a masking agent for the analysis of aluminum in the presence of iron.

Solution

A suitable masking agent must form a complex with the interferent, but not with the analyte. Oxalate, for example, is not a suitable masking agent because it binds both Al and Fe. Thioglycolic acid, on the other hand, is a selective masking agent for Fe in the presence of Al. Other acceptable masking agents are cyanide (CN^-) thiocyanate (SCN^-), and thiosulfate ($S_2O_3^{2-}$).

? Exercise 7.6.1

Using Table 7.6, suggest a masking agent for the analysis of Fe in the presence of Al.

Answer

The fluoride ion, F^-, is a suitable masking agent as it binds with Al^{3+} to form the stable AlF_6^{3-} complex, leaving iron in solution.

As shown in Example 7.6.2 , we can judge a masking agent's effectiveness by considering the relevant equilibrium constants.

✔ Example 7.6.2

Show that CN^- is an appropriate masking agent for Ni^{2+} in a method where nickel's complexation with EDTA is an interference.

Solution

The relevant reactions and formation constants are

$$Ni^{2+}(aq) + Y^{4-}(aq) \rightleftharpoons NiY^{2-}(aq) \quad K_1 = 4.2 \times 10^{18}$$

$$Ni^{2+}(aq) + 4CN^-(aq) \rightleftharpoons Ni(CN)_4^{2-}(aq) \quad \beta_4 = 1.7 \times 10^{30}$$

where Y^{4-} is an abbreviation for EDTA. Cyanide is an appropriate masking agent because the formation constant for $Ni(CN)_4^{2-}$ is greater than that for the Ni–EDTA complex. In fact, the equilibrium constant for the reaction in which EDTA displaces the masking agent

$$Ni(CN)_4^{2-}(aq) + Y^{4-}(aq) \rightleftharpoons NiY^{2-}(aq) + 4CN^-(aq)$$

$$K = \frac{K_1}{\beta_4} = \frac{4.2 \times 10^{18}}{1.7 \times 10^{30}} = 2.5 \times 10^{-12}$$

is sufficiently small that $Ni(CN)_4^{2-}$ is relatively inert in the presence of EDTA.

? Exercise 7.6.2

Use the formation constants in Appendix 12 to show that 1,10-phenanthroline is a suitable masking agent for Fe^{2+} in the presence of Fe^{3+}. Use a ladder diagram to define any limitations on using 1,10-phenanthroline as a masking agent. See Chapter 6 for a review of ladder diagrams.

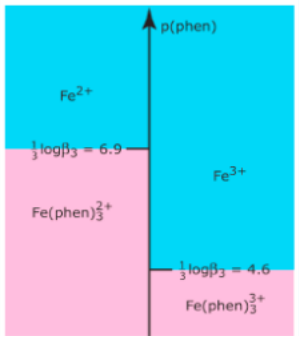
Answer

The relevant reactions and equilibrium constants are

$$Fe^{2+}(aq) + 3phen(aq) \rightleftharpoons Fe(phen)_3^{2+}(aq) \quad \beta_3 = 5 \times 10^{20}$$

$$Fe^{3+}(aq) + 3phen(aq) \rightleftharpoons Fe(phen)_3^{3+}(aq) \quad \beta_3 = 6 \times 10^{13}$$

where phen is an abbreviation for 1,10-phenanthroline. Because β_3 is larger for the complex with Fe^{2+} than it is for the complex with Fe^{3+},1,10-phenanthroline will bind Fe^{2+} before it binds Fe^{3+}. A ladder diagram for this system (as shown below) suggests that an equilibrium p(phen) between 5.6 and 5.9 will fully complex Fe^{2+} without any significant formation of the $Fe(phen)_3^{3+}$ complex. Adding a stoichiometrically equivalent amount of 1,10-phenanthroline to a solution of Fe^{2+} is sufficient to mask Fe^{2+} in the presence of Fe^{3+}. A large excess of 1,10-phenanthroline, however, decreases p(phen) and allows for the formation of both metal–ligand complexes.



Separations Based on a Change of State

Because an analyte and its interferent are usually in the same phase, we can achieve a separation if one of them undergoes a change in its physical state or its chemical state.

Changes in Physical State

When the analyte and the interferent are miscible liquids, separation by ***distillation*** is possible if their boiling points are significantly different. Figure 7.6.5 shows the progress of a distillation as a plot of temperature versus the composition of mixture's vapor-phase and liquid-phase. The initial liquid mixture (point A), contains more interferent than analyte. When this solution is brought to its boiling point, the vapor phase in equilibrium with the liquid phase is enriched in analyte (point B). The horizontal line that connects points A and B represents this vaporization equilibrium. Condensing the vapor phase at point B, by lowering the temperature, creates a new liquid phase with a composition identical to that in the vapor phase (point C). The vertical line that connects points B and C represents this condensation equilibrium. The liquid phase at point C has a lower boiling point than the original mixture, and is in equilibrium with the vapor phase at point D. This process of repeated vaporization and condensation gradually separates the analyte and the interferent.

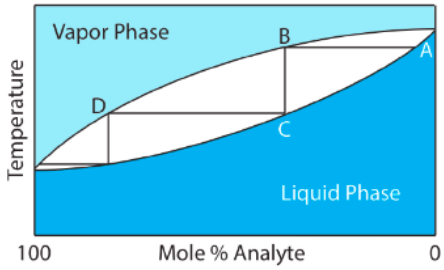


Figure 7.6.5 . Boiling point versus composition diagram for a near-ideal solution consisting of a low-boiling analyte and a high-boiling interferent. The horizontal lines represent vaporization equilibria and the vertical lines represent condensation equilibria. See the text for additional details.

Two experimental set-ups for distillations are shown in Figure 7.6.6 . The simple distillation apparatus shown in Figure 7.6.6 a is useful only for separating a volatile analyte (or interferent) from a non-volatile interferent (or analyte), or for separating an analyte and an interferent whose boiling points differ by more than 150°C. A more efficient separation is achieved using the fractional distillation apparatus in Figure 7.6.6 b. Packing the fractionating column with a high surface area material, such as a steel sponge or glass beads, provides more opportunity for the repeated process of vaporization and condensation necessary to effect a complete separation.

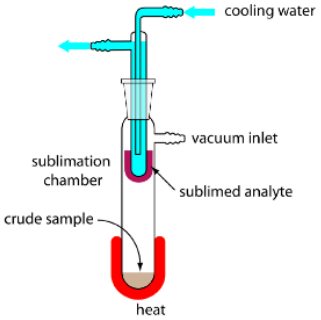


Figure 7.6.7 . Typical experimental set-up for a sublimation. The sample is placed in the sublimation chamber, which can be evacuated. Heating the sample causes the analyte to vaporize and sublime onto the cold finger, which is cooled using cold water.

Recrystallization is another method for purifying a solid. A solvent is chosen in which the analyte's solubility is significant when the solvent is hot and minimal when the solvent is cold. The interferents must be less soluble in the hot solvent than the analyte or present in much smaller amounts. After heating a portion of the solvent in an Erlenmeyer flask, small amounts of sample are added until undissolved sample is visible. Additional hot solvent is added until the sample redissolves, or until only insoluble impurities remain. This process of adding sample and solvent is repeated until the entire sample is added to the Erlenmeyer flask. Any insoluble impurities are removed by filtering the hot solution. The solution is allowed to cool slowly, which promotes the growth of large, pure crystals, and then cooled in an ice bath to minimize solubility losses. The purified sample is isolated by filtration and rinsed to remove any soluble impurities. Finally, the sample is dried to remove any remaining traces of the solvent. Further purification, if necessary, is accomplished by additional recrystallizations.

Changes in Chemical State

Distillation, sublimation, and recrystallization use a change in physical state to effect a separation. Chemical reactivity also is a useful tool for separating analytes and interferents. For example, we can separate SiO_2 from a sample by reacting it with HF to form SiF_4. Because SiF_4 is volatile, it is easy to remove by evaporation. If we wish to collect the reaction's volatile product, then a distillation is possible. For example, we can isolate the NH_4^+ in a sample by making the solution basic and converting it to NH_3. The ammonia is then removed by distillation. Table 7.6.4 provides additional examples of this approach for isolating inorganic ions.

Table 7.6.4 . Examples of Using a Chemical Reaction and a Distillation to Separate an Inorganic Analyte From Interferents

analyte	treatment	isolated species
CO_3^{2-}	$CO_3^{2-}(aq) + 2H_3O^+(aq) \rightarrow CO_2(g) + 3H_2O(l)$	CO_2
NH_4^+	$NH_4^+(aq) + OH^-(aq) \rightarrow NH_3(aq) + H_2O(l)$	NH_3
SO_3^-	$SO_3^{2-}(aq) + 2H_3O^+(aq) \rightarrow SO_2(g) + 3H_2O(l)$	SO_2
S^{2-}	$S^{2-}(aq) + 2H_3O^+(aq) \rightarrow H_2S(g) + 2H_2O(l)$	H_2S

Another reaction for separating analytes and interferents is precipitation. Two important examples of using a precipitation reaction in a separation are the pH-dependent solubility of metal oxides and hydroxides, and the pH-dependent solubility of metal sulfides.

Separations based on the pH-dependent solubility of oxides and hydroxides usually use a strong acid, a strong base, or an NH_3/NH_4Cl buffer to adjust the pH. Most metal oxides and hydroxides are soluble in hot concentrated HNO_3, although a few oxides, such as WO_3, SiO_2, and SnO_2 remain insoluble even under these harsh conditions. To determine the amount of Cu in brass, for example, we can avoid an interference from Sn by dissolving the sample with a strong acid and filtering to remove the solid residue of SnO_2.

Most metals form a hydroxide precipitate in the presence of concentrated NaOH. Those metals that form amphoteric hydroxides, however, do not precipitate because they react to form higher-order hydroxo-complexes. For example, Zn^{2+} and Al^{3+} do not precipitate in concentrated NaOH because they form the soluble complexes $\mathbf{Zn(OH)_3^-}$ and $\mathbf{Al(OH)_4^-}$. The solubility of Al^{3+} in concentrated NaOH allows us to isolate aluminum from impure samples of bauxite, an ore of Al_2O_3. After crushing the ore, we place it in a solution of concentrated NaOH, dissolving the Al_2O_3 and forming $\mathbf{Al(OH)_4^-}$. Other oxides in the ore, such as Fe_2O_3 and SiO_2, remain insoluble. After filtering, we recover the aluminum as a precipitate of $Al(OH)_3$ by neutralizing some of the OH^- with acid.

The pH of an NH_3/NH_4Cl buffer (pK_a = 9.26) is sufficient to precipitate most metals as the hydroxide. The alkaline earths and alkaline metals, however, do not precipitate at this pH. In addition, metal ions that form soluble complexes with NH_3, such as Cu^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+} also do not precipitate under these conditions.

The use of S^{2-} as a precipitating reagent is one of the earliest examples of a separation technique. In Fresenius's 1881 text *A System of Instruction in Quantitative Chemical Analysis*, sulfide frequently is used to separate metal ions from the remainder of the sample's matrix [Fresenius. C. R. *A System of Instruction in Quantitative Chemical Analysis*, John Wiley and Sons: New York, 1881]. Sulfide is a useful reagent for separating metal ions for two reasons: (1) most metal ions, except for the alkaline earths and alkaline metals, form insoluble sulfides; and (2) these metal sulfides show a substantial variation in solubility. Because the concentration of S^{2-} is pH-dependent, we can control which metal ions precipitate by adjusting the pH. For example, in Fresenius's gravimetric procedure for the determination of Ni in ore samples (see Figure 1.1.1 for a schematic diagram of this procedure), sulfide is used three times to separate Co^{2+} and Ni^{2+} from Cu^{2+} and, to a lesser extent, from Pb^{2+}.

Separations Based on a Partitioning Between Phases

The most important group of separation techniques uses a selective partitioning of the analyte or interferent between two immiscible phases. If we bring a phase that contains the solute, *S*, into contact with a second phase, the solute will partition itself between the two phases, as shown by the following equilibrium reaction.

$$S_{\text{phase 1}} \rightleftharpoons S_{\text{phase 2}} \tag{7.6.1}$$

The equilibrium constant for reaction 7.6.1

$$K_D = \frac{[S_{\text{phase 2}}]}{[S_{\text{phase 1}}]}$$

is called the distribution constant or the ***partition coefficient***. If K_D is sufficiently large, then the solute moves from phase 1 to phase 2. The solute will remain in phase 1 if the partition coefficient is sufficiently small. When we bring a phase that contains two solutes into contact with a second phase, a separation of the solutes is possible if K_D is favorable for only one of the solutes. The physical states of the phases are identified when we describe the separation process, with the phase that contains the sample listed first. For example, if the sample is in a liquid phase and the second phase is a solid, then the separation involves liquid–solid partitioning.

Extraction Between Two Phases

We call the process of moving a species from one phase to another phase an ***extraction***. Simple extractions are particularly useful for separations where only one component has a favorable partition coefficient. Several important separation techniques are based on a simple extraction, including liquid–liquid, liquid–solid, solid–liquid, and gas–solid extractions.

Liquid-Liquid Extractions

A liquid–liquid extraction usually is accomplished using a separatory funnel (Figure 7.6.8). After placing the two liquids in the separatory funnel, we shake the funnel to increase the surface area between the phases. When the extraction is complete, we allow the liquids to separate. The stopcock at the bottom of the separatory funnel allows us to remove the two phases.

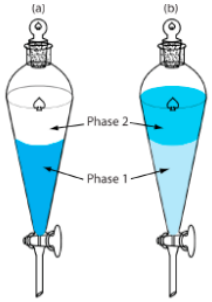


Figure 7.6.8 . Example of a liquid–liquid extraction using a separatory funnel. (a) Before the extraction, 100% of the analyte is in phase 1. (b) After the extraction, most of the analyte is in phase 2, although some analyte remains in phase 1. Although one liquid–liquid extraction can result in the complete transfer of analyte, a single extraction usually is not sufficient. See Chapter7.7 for a discussion of extraction efficiency and multiple extractions.

We also can carry out a liquid–liquid extraction without a separatory funnel by adding the extracting solvent to the sample's container. Pesticides in water, for example, are preserved in the field by extracting them into a small volume of hexane. A liquid–liquid microextraction, in which the extracting phase is a 1-μL drop suspended from a microsyringe (Figure 7.6.9), also has been described [Jeannot, M. A.; Cantwell, F. F. *Anal. Chem.* **1997**, *69*, 235–239]. Because of its importance, a more thorough discussion of liquid–liquid extractions is in Chapter7.7.

Solid Phase Extractions

In a solid phase extraction of a liquid sample, we pass the sample through a cartridge that contains a solid adsorbent, several examples of which are shown in Figure 7.6.10 . The choice of adsorbent is determined by the species we wish to separate. Table 7.6.5 provides several representative examples of solid adsorbents and their applications.

Figure 7.6.10 . Selection of solid phase extraction cartridges for liquid samples. The solid adsorbent is the white or black material in each cartridge. From left-to-right, the absorbent materials are octadecylsilane, carbon, octadecylsilane, polyamide resin, and diol; see Table 7.6.5 for additional details. The size of the cartridges dictates the volume of sample used; from left-to-right, these cartridges use samples of 1 mL, 3 mL, 6 mL, 3 mL, and 1 mL.

Table 7.6.5 . Representative Adsorbents for the Solid Phase Extraction of Liquid Samples

absorbent	structure	properties and uses
silica	—Si—OH	• retains low to moderate polarity species from organic matrices • fat soluble vitamins, steroids
aminopropyl	—Si— NH_2	• retains polar compounds • carbohydrates, organic acids
cyanopropyl	—Si— CN	• retains wide variety of species from aqueous and organic matrices • pesticides, hydrophobic peptides
diol	—Si— OH, OH	• retains wide variety of species from aqueous and organic matrices • proteins, peptides, fungicides
octadecyl (C–18)	$—C_{18}H_{37}$	• retains hydrophobic species from aqueous matrices • caffeine, sedatives, polyaromatic hydrocarbons carbohydrates, pesticides
octyl (C–8)	$—C_8H_{17}$	• similar to C-18

As an example, let's examine a procedure for isolating the sedatives secobarbital and phenobarbital from serum samples using a C-18 solid adsorbent [Alltech Associates *Extract-Clean SPE Sample Preparation Guide*, Bulletin 83]. Before adding the sample, the solid phase cartridge is rinsed with 6 mL each of methanol and water. Next, a 500-µL sample of serum is pulled through the cartridge, with the sedatives and matrix interferents retained following a liquid–solid extraction (Figure 7.6.11 a). Washing the cartridge with distilled water removes any interferents (Figure 7.6.11 b). Finally, we elute the sedatives using 500 µL of acetone (Figure 7.6.11 c). In comparison to a liquid–liquid extraction, a solid phase extraction has the advantage of being easier, faster, and requires less solvent.

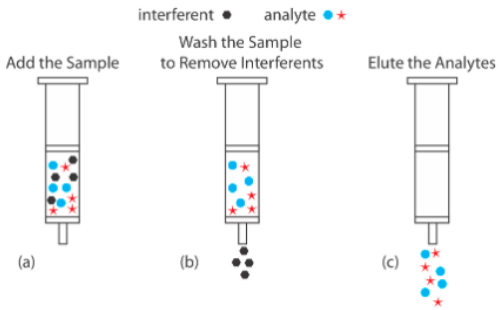


Figure 7.6.11 . Steps in a typical solid phase extraction. After preconditioning the solid phase cartridge with solvent, (a) the sample is added to the cartridge, (b) the sample is washed to remove interferents, and (c) the analytes are eluted.

Continuous Extractions

An extraction is possible even if the analyte has an unfavorable partition coefficient, provided that the sample's other components have significantly smaller partition coefficients. Because the analyte's partition coefficient is unfavorable, a single extraction will not recover all the analyte. Instead we continuously pass the extracting phase through the sample until we achieve a quantitative extraction.

A continuous extraction of a solid sample is carried out using a ***Soxhlet extractor*** (Figure 7.6.12). The extracting solvent is placed in the lower reservoir and heated to its boiling point. Solvent in the vapor phase moves upward through the tube on the far right side of the apparatus, reaching the condenser where it condenses back to the liquid state. The solvent then passes through the sample, which is held in a porous cellulose filter thimble, collecting in the upper reservoir. When the solvent in the upper reservoir reaches the return tube's upper bend, the solvent and extracted analyte are siphoned back to the lower reservoir. Over time the analyte's concentration in the lower reservoir increases.

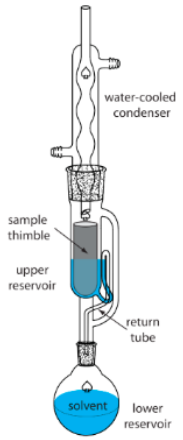


Figure 7.6.12 . Soxhlet extractor. See text for details.

Microwave-assisted extractions have replaced Soxhlet extractions in some applications [Renoe, B. W. *Am. Lab* August **1994**, 34–40]. The process is the same as that described earlier for a microwave digestion. After placing the sample and the solvent in a sealed digestion vessel, a microwave oven is used to heat the mixture. Using a sealed digestion vessel allows the extraction to take place at a higher temperature and pressure, reducing the amount of time needed for a quantitative extraction. In a Soxhlet extraction the temperature is limited by the solvent's boiling point at atmospheric pressure. When acetone is the solvent, for example, a Soxhlet extraction is limited to 56°C, but a microwave extraction can reach 150°C.

Two other continuous extractions deserve mention. Volatile organic compounds (VOCs) can be quantitatively removed from a liquid sample by a liquid–gas extraction. As shown in Figure 7.6.13 , an inert purging gas, such as He, is passed through the sample. The purge gas removes the VOCs, which are swept to a primary trap where they collect on a solid absorbent. When the extraction is complete, the VOCs are removed from the primary trap by rapidly heating the tube while flushing with He. This technique is known as a ***purge-and-trap***. Because the analyte's recovery may not be reproducible, an internal standard is required for quantitative work.

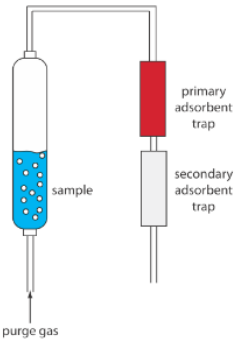


Figure 7.6.13 . Schematic diagram of a purge-and-trap system for extracting volatile analytes. The purge gas releases the analytes, which subsequently collect in the primary adsorbent trap. The secondary adsorption trap is monitored for evidence that the primary trap's capacity to absorb analyte is exceeded.

Continuous extractions also can be accomplished using supercritical fluids [McNally, M. E. *Anal. Chem.* **1995**, *67*, 308A–315A]. If we heat a substance above its critical temperature and pressure it forms a ***supercritical fluid*** whose properties are between those of a gas and a liquid. A supercritical fluid is a better solvent than a gas, which makes it a better reagent for extractions. In addition, a supercritical fluid's viscosity is significantly less than that of a liquid, which makes it easier to push it through a particulate sample. One example of a supercritical fluid extraction is the determination of total petroleum hydrocarbons (TPHs) in soils, sediments, and sludges using supercritical CO_2 ["TPH Extraction by SFE," ISCO, Inc. Lincoln, NE, Revised Nov. 1992]. An approximately 3-g sample is placed in a 10-mL stainless steel cartridge and supercritical CO_2 at a pressure of 340 atm and a temperature of 80°C is passed through the cartridge for 30 minutes at flow rate of 1–2 mL/min. To collect the TPHs, the effluent from the cartridge is passed through 3 mL of tetrachloroethylene at room temperature. At this temperature the CO_2 reverts to the gas phase and is released to the atmosphere.

Chromatographic Separations

In an extraction, the sample originally is in one phase and we extract the analyte or the interferent into a second phase. We also can separate the analyte and interferents by continuously passing one sample-free phase, called the mobile phase, over a second sample-free phase that remains fixed or stationary. The sample is injected into the mobile phase and the sample's components partition themselves between the mobile phase and the stationary phase. Those components with larger partition coefficients are more likely to move into the stationary phase and take longer time to pass through the system. This is the basis of all chromatographic separations. Chromatography provides both a separation of analytes and interferents, and a means for performing a qualitative or quantitative analysis for the analyte. For this reason a more thorough treatment of chromatography is found in Chapter 12.

7.7: Liquid-Liquid Extractions

A liquid–liquid extraction is an important separation technique for environmental, clinical, and industrial laboratories. A standard environmental analytical method illustrates the importance of liquid–liquid extractions. Municipal water departments routinely monitor public water supplies for trihalomethanes ($CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$) because they are known or suspected carcinogens. Before their analysis by gas chromatography, trihalomethanes are separated from their aqueous matrix using a liquid–liquid extraction with pentane ["The Analysis of Trihalomethanes in Drinking Water by Liquid Extraction,"EPAMethod501.2 (EPA 500-Series, November 1979)].

> The Environmental Protection Agency (EPA) also publishes two additional methods for trihalomethanes. Method 501.1 and Method 501.3 use a purge-and-trap to collect the trihalomethanes prior to a gas chromatographic analysis with a halide-specific detector (Method 501.1) or a mass spectrometer as the detector (Method 501.3). You will find more details about gas chromatography, including detectors, in Chapter 12.

In a simple liquid–liquid extraction the solute partitions itself between two immiscible phases. One phase usually is an aqueous solvent and the other phase is an organic solvent, such as the pentane used to extract trihalomethanes from water. Because the phases are immiscible they form two layers, with the denser phase on the bottom. The solute initially is present in one of the two phases; after the extraction it is present in both phases. ***Extraction efficiency***—that is, the percentage of solute that moves from one phase to the other—is determined by the equilibrium constant for the solute's partitioning between the phases and any other side reactions that involve the solute. Examples of other reactions that affect extraction efficiency include acid–base reactions and complexation reactions.

Partition Coefficients and Distribution Ratios

As we learned earlier in this chapter, a solute's partitioning between two phases is described by a partition coefficient, K_D. If we extract a solute from an aqueous phase into an organic phase

$$S_{aq} \rightleftharpoons S_{org}$$

then the partition coefficient is

$$K_D = \frac{[S_{org}]}{[S_{aq}]}$$

A large value for K_D indicates that extraction of solute into the organic phase is favorable.

To evaluate an extraction's efficiency we must consider the solute's total concentration in each phase, which we define as a ***distribution ratio***, D.

$$D = \frac{[S_{org}]_{\text{total}}}{[S_{aq}]_{\text{total}}}$$

The partition coefficient and the distribution ratio are identical if the solute has only one chemical form in each phase; however, if the solute exists in more than one chemical form in either phase, then K_D and D usually have different values. For example, if the solute exists in two forms in the aqueous phase, A and B, only one of which, A, partitions between the two phases, then

$$D = \frac{[S_{org}]_A}{[S_{aq}]_A + [S_{aq}]_B} \leq K_D = \frac{[S_{org}]_A}{[S_{aq}]_A}$$

This distinction between K_D and D is important. The partition coefficient is a thermodynamic equilibrium constant and has a fixed value for the solute's partitioning between the two phases. The distribution ratio's value, however, changes with solution conditions if the relative amounts of A and B change. If we know the solute's equilibrium reactions within each phase and between the two phases, we can derive an algebraic relationship between K_D and D.

Liquid-Liquid Extraction With No Secondary Reactions

In a simple liquid–liquid extraction, the only reaction that affects the extraction efficiency is the solute's partitioning between the two phases (Figure 7.7.1).

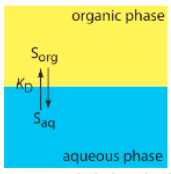


Figure 7.7.1 . Scheme for a simple liquid–liquid extraction in which the solute's partitioning depends only on the K_D equilibrium.

In this case the distribution ratio and the partition coefficient are equal.

$$D = \frac{[S_{org}]_{\text{total}}}{[S_{aq}]_{\text{total}}} = K_D = \frac{[S_{org}]}{[S_{aq}]} \tag{7.7.1}$$

Let's assume the solute initially is present in the aqueous phase and that we wish to extract it into the organic phase. A conservation of mass requires that the moles of solute initially present in the aqueous phase equal the combined moles of solute in the aqueous phase and the organic phase after the extraction.

$$(\text{mol } S_{aq})_0 = (\text{mol } S_{aq})_1 + (\text{mol } S_{org})_1 \tag{7.7.2}$$

where the subscripts indicate the extraction number with 0 representing the system before the extraction and 1 the system following the first extraction. After the extraction, the solute's concentration in the aqueous phase is

$$[S_{aq}]_1 = \frac{(\text{mol } S_{aq})_1}{V_{aq}} \tag{7.7.3}$$

and its concentration in the organic phase is

$$[S_{org}]_1 = \frac{(\text{mol } S_{org})_1}{V_{org}} \tag{7.7.4}$$

where V_{aq} and V_{org} are the volumes of the aqueous phase and the organic phase. Solving Equation 7.7.2 for $(\text{mol } S_{org})_1$ and substituting into Equation 7.7.4 leave us with

$$[S_{org}]_1 = \frac{(\text{mol } S_{aq})_0 - (\text{mol } S_{aq})_1}{V_{org}} \tag{7.7.5}$$

Substituting Equation 7.7.3 and Equation 7.7.5 into Equation 7.7.1 gives

$$D = \frac{\frac{(\text{mol } S_{aq})_0 - (\text{mol } S_{aq})_1}{V_{org}}}{\frac{(\text{mol } S_{aq})_1}{V_{aq}}} = \frac{(\text{mol } S_{aq})_0 \times V_{aq} - (\text{mol } S_{aq})_1 \times V_{aq}}{(\text{mol } S_{aq})_1 \times V_{org}}$$

Rearranging and solving for the fraction of solute that remains in the aqueous phase after one extraction, $(q_{aq})_1$, gives

$$(q_{aq})_1 = \frac{(\text{mol } S_{aq})_1}{(\text{mol } S_{aq})_0} = \frac{V_{aq}}{DV_{org} + V_{aq}} \tag{7.7.6}$$

The fraction present in the organic phase after one extraction, $(q_{org})_1$, is

$$(q_{org})_1 = \frac{(\text{mol } S_{org})_1}{(\text{mol } S_{aq})_0} = 1 - (q_{aq})_1 = \frac{DV_{org}}{DV_{org} + V_{aq}}$$

Example 7.7.1 shows how we can use Equation 7.7.6 to calculate the efficiency of a simple liquid-liquid extraction.

✔ Example 7.7.1

A solute has a K_D between water and chloroform of 5.00. Suppose we extract a 50.00-mL sample of a 0.050 M aqueous solution of the solute using 15.00 mL of chloroform. (a) What is the separation's extraction efficiency? (b) What volume of chloroform do we need if we wish to extract 99.9% of the solute?

Solution

For a simple liquid–liquid extraction the distribution ratio, D, and the partition coefficient, K_D, are identical.

(a) The fraction of solute that remains in the aqueous phase after the extraction is given by Equation 7.7.6.

$$(q_{aq})_1 = \frac{V_{aq}}{DV_{org} + V_{aq}} = \frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}} = 0.400$$

The fraction of solute in the organic phase is 1–0.400, or 0.600. Extraction efficiency is the percentage of solute that moves into the extracting phase; thus, the extraction efficiency is 60.0%.

(b) To extract 99.9% of the solute $(q_{aq})_1$ must be 0.001. Solving Equation 7.7.6 for V_{org}, and making appropriate substitutions for $(q_{aq})_1$ and V_{aq} gives

$$V_{org} = \frac{V_{aq} - (q_{aq})_1 V_{aq}}{(q_{aq})_1 D} = \frac{50.00 \text{ mL} - (0.001)(50.00 \text{ mL})}{(0.001)(5.00 \text{ mL})} = 999 \text{ mL}$$

This is large volume of chloroform. Clearly, a single extraction is not reasonable under these conditions.

In Example 7.7.1 , a single extraction provides an extraction efficiency of only 60%. If we carry out a second extraction, the fraction of solute remaining in the aqueous phase, $(q_{aq})_2$, is

$$(q_{aq})_2 = \frac{(\text{mol } S_{aq})_2}{(\text{mol } S_{aq})_1} = \frac{V_{aq}}{DV_{org} + V_{aq}}$$

If V_{aq} and V_{org} are the same for both extractions, then the cumulative fraction of solute that remains in the aqueous layer after two extractions, $(Q_{aq})_2$, is the product of $(q_{aq})_1$ and $(q_{aq})_2$, or

$$(Q_{aq})_2 = \frac{(\text{mol } S_{aq})_2}{(\text{mol } S_{aq})_0} = (q_{aq})_1 \times (q_{aq})_2 = \left(\frac{V_{aq}}{DV_{org} + V_{aq}}\right)^2$$

In general, for a series of n identical extractions, the fraction of analyte that remains in the aqueous phase after the last extraction is

$$(Q_{aq})_n = \left(\frac{V_{aq}}{DV_{org} + V_{aq}}\right)^n \tag{7.7.7}$$

✔ Example 7.7.2

For the extraction described in Example 7.7.1 , determine (a) the extraction efficiency for two identical extractions and for three identical extractions; and (b) the number of extractions required to ensure that we extract 99.9% of the solute.

Solution

(a) The fraction of solute remaining in the aqueous phase after two extractions and three extractions is

$$(Q_{aq})_2 = \left(\frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}}\right)^2 = 0.160$$

$$(Q_{aq})_3 = \left(\frac{50.0 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}}\right)^3 = 0.0640$$

The extraction efficiencies are 84.0% for two extractions and 93.6% for three extractions.

(b) To determine the minimum number of extractions for an efficiency of 99.9%, we set $(Q_{aq})_n$ to 0.001 and solve for n using Equation 7.7.7.

$$0.001 = \left(\frac{50.00 \text{ mL}}{(5.00)(15.00 \text{ mL}) + 50.00 \text{ mL}}\right)^n = (0.400)^n$$

Taking the log of both sides and solving for n

$$\log(0.001) = n \log(0.400)$$
$$n = 7.54$$

we find that a minimum of eight extractions is necessary.

? Exercise 7.7.1

To plan a liquid–liquid extraction we need to know the solute's distribution ratio between the two phases. One approach is to carry out the extraction on a solution that contains a known amount of solute. After the extraction, we isolate the organic phase and allow it to evaporate, leaving behind the solute. In one such experiment, 1.235 g of a solute with a molar mass of 117.3 g/mol is dissolved in 10.00 mL of water. After extracting with 5.00 mL of toluene, 0.889 g of the solute is recovered in the organic phase. (a) What is the solute's distribution ratio between water and toluene? (b) If we extract 20.00 mL of an aqueous solution that contains the solute using 10.00 mL of toluene, what is the extraction efficiency? (c) How many extractions will we need to recover 99.9% of the solute?

Answer

(a) The solute's distribution ratio between water and toluene is

$$D = \frac{[S_{org}]}{[S_{aq}]} = \frac{0.889\text{ g} \times \frac{1\text{ mol}}{117.3\text{ g}} \times \frac{1}{0.00500\text{ L}}}{(1.235\text{ g} - 0.889\text{ g}) \times \frac{1\text{ mol}}{117.3\text{ g}} \times \frac{1}{0.01000\text{ L}}} = 5.14$$

(b) The fraction of solute remaining in the aqueous phase after one extraction is

$$(q_{aq})_1 = \frac{V_{aq}}{DV_{org} + V_{aq}} = \frac{20.00\text{ mL}}{(5.14)(10.00\text{ mL}) + 20.00\text{ mL}} = 0.280$$

The extraction efficiency, therefore, is 72.0%.

(c) To extract 99.9% of the solute requires

$$(Q_{aq})_n = 0.001 = \left(\frac{20.00\text{ mL}}{(5.14)(10.00\text{ mL}) + 20.00\text{ mL}}\right)^n = (0.280)^n$$

$$\log(0.001) = n\log(0.280)$$

$$n = 5.4$$

a minimum of six extractions.

Liquid-Liquid Extractions Involving Acid-Base Equilibria

As we see in Equation 7.7.1, in a simple liquid–liquid extraction the distribution ratio and the partition coefficient are identical. As a result, the distribution ratio does not depend on the composition of the aqueous phase or the organic phase. A change in the pH of the aqueous phase, for example, will not affect the solute's extraction efficiency when K_D and D have the same value.

If the solute participates in one or more additional equilibrium reactions within a phase, then the distribution ratio and the partition coefficient may not be the same. For example, Figure 7.7.3 shows the equilibrium reactions that affect the extraction of the weak acid, HA, by an organic phase in which ionic species are not soluble.

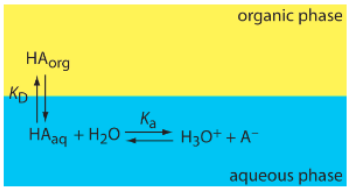


Figure 7.7.3 . Scheme for the liquid–liquid extraction of a weak acid, HA. Although the weak acid is soluble in both phases, its conjugate weak base, A^-, is soluble in the aqueous phase only. The K_a reaction for HA, which is called a ***secondary equilibrium reaction***, affects weak acid's extraction efficiency because it determines the relative abundance of HA in solution.

In this case the partition coefficient and the distribution ratio are

$$K_D = \frac{[HA_{org}]}{[HA_{aq}]} \tag{7.7.8}$$

$$D = \frac{[HA_{org}]_{total}}{[HA_{aq}]_{total}} = \frac{[HA_{org}]}{[HA_{aq}] + [A^-_{aq}]} \tag{7.7.9}$$

Because the position of an acid–base equilibrium depends on pH, the distribution ratio, D, is pH-dependent. To derive an equation for D that shows this dependence, we begin with the acid dissociation constant for HA.

$$K_a = \frac{[H_3O^+_{aq}]\,[A^-_{aq}]}{[HA_{aq}]} \tag{7.7.10}$$

Solving Equation 7.7.10 for the concentration of A^- in the aqueous phase

$$[A^-_{aq}] = \frac{K_a \times [HA_{aq}]}{[H_3O^+_{aq}]}$$

and substituting into Equation 7.7.9 gives

$$D = \frac{[HA_{org}]}{[HA_{aq}] + \frac{K_a \times [HA_{aq}]}{[H_3O^+_{aq}]}}$$

Factoring $[HA_{aq}]$ from the denominator, replacing $[HA_{org}]/[HA_{aq}]$ with K_D (Equation 7.7.8), and simplifying leaves us with the following relationship between the distribution ratio, D, and the pH of the aqueous solution.

$$D = \frac{K_D\,[H_3O^+_{aq}]}{[H_3O^+_{aq}] + K_a} \tag{7.7.11}$$

Example 7.7.3

An acidic solute, HA, has a K_a of 1.00×10^{-5} and a K_D between water and hexane of 3.00. Calculate the extraction efficiency if we extract a 50.00 mL sample of a 0.025 M aqueous solution of HA, buffered to a pH of 3.00, with 50.00 mL of hexane. Repeat for pH levels of 5.00 and 7.00.

Solution

When the pH is 3.00, $[H_3O^+_{aq}]$ is 1.0×10^{-3} and the distribution ratio is

$$D = \frac{(3.00)\left(1.0 \times 10^{-3}\right)}{1.0 \times 10^{-3} + 1.00 \times 10^{-5}} = 2.97$$

The fraction of solute that remains in the aqueous phase is

$$(Q_{aq})_1 = \frac{50.00\ \text{mL}}{(2.97)(50.00\ \text{mL}) + 50.00\ \text{mL}} = 0.252$$

The extraction efficiency, therefore, is almost 75%. The same calculation at a pH of 5.00 gives the extraction efficiency as 60%. At a pH of 7.00 the extraction efficiency is just 3% .

The extraction efficiency in Example 7.7.3 is greater at more acidic pH levels because HA is the solute's predominate form in the aqueous phase. At a more basic pH, where A^- is the solute's predominate form, the extraction efficiency is smaller. A graph of extraction efficiency versus pH is shown in Figure 7.7.4 . Note that extraction efficiency essentially is independent of pH for pH levels more acidic than the HA's pK_a, and that it is essentially zero for pH levels more basic than HA's pK_a. The greatest change in extraction efficiency occurs at pH levels where both HA and A^- are predominate species. The ladder diagram for HA along the graph's *x*-axis helps illustrate this effect.

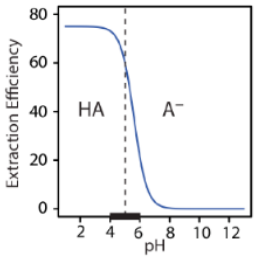


Figure 7.7.4 . Plot of extraction efficiency versus pH of the aqueous phase for the extraction in Example 7.7.3 . A ladder diagram for HA is superimposed along the *x*-axis, which divides the pH scale into regions where HA and A^- are the predominate aqueous phase species. The greatest change in extraction efficiency occurs as the pH moves through HA's buffer region.

Exercise 7.7.2

The liquid–liquid extraction of the weak base B is governed by the following equilibrium reactions:

$$B(aq) \rightleftharpoons B(org) \quad K_D = 5.00$$
$$B(aq) + H_2O(l) \rightleftharpoons OH^-(aq) + HB^+(aq) \quad K_b = 1.0 \times 10^{-4}$$

Derive an equation for the distribution ratio, *D*, and calculate the extraction efficiency if 25.0 mL of a 0.025 M solution of B, buffered to a pH of 9.00, is extracted with 50.0 mL of the organic solvent.

Answer

Liquid-Liquid Extraction of a Metal-Ligand Complex

One important application of a liquid–liquid extraction is the selective extraction of metal ions using an organic ligand. Unfortunately, many organic ligands are not very soluble in water or undergo hydrolysis or oxidation reactions in aqueous solutions. For these reasons the ligand is added to the organic solvent instead of the aqueous phase. Figure 7.7.5 shows the relevant equilibrium reactions (and equilibrium constants) for the extraction of M^{n+} by the ligand HL, including the ligand's extraction into the aqueous phase ($K_{D,HL}$), the ligand's acid dissociation reaction (K_a), the formation of the metal–ligand complex (β_n), and the complex's extraction into the organic phase ($K_{D,c}$).

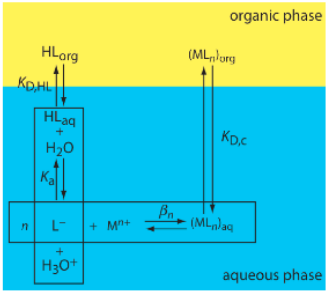


Figure 7.7.5 . Scheme for the liquid–liquid extraction of a metal ion, M^{n+}, by the ligand L^-. The ligand initially is present in the organic phase as HL. Four equilibrium reactions are needed to explain the extraction efficiency.

If the ligand's concentration is much greater than the metal ion's concentration, then the distribution ratio is

$$D = \frac{\beta_n K_{D,c} (K_a)^n (C_{HL})^n}{(K_{D,HL})^n [H_3O^+]^n + \beta_n (K_a)^n (C_{HL})^n} \tag{7.7.12}$$

where C_{HL} is the ligand's initial concentration in the organic phase. As shown in Example 7.7.4 , the extraction efficiency for metal ions shows a marked pH dependency.

Example 7.7.4

A liquid–liquid extraction of the divalent metal ion, M^{2+}, uses the scheme outlined in Figure 7.7.5 . The partition coefficients for the ligand, $K_{D,HL}$, and for the metal–ligand complex, $K_{D,c}$, are 1.0×10^4 and 7.0×10^4, respectively. The ligand's acid dissociation constant, K_a, is 5.0×10^{-5}, and the formation constant for the metal–ligand complex, β_2, is 2.5×10^{16}. What is the extraction efficiency if we extract 100.0 mL of a 1.0×10^{-6} M aqueous solution of M^{2+}, buffered to a pH of 1.00, with 10.00 mL of an organic solvent that is 0.1 mM in the chelating agent? Repeat the calculation at a pH of 3.00.

Solution

When the pH is 1.00 the distribution ratio is

$$D = \frac{(2.5 \times 10^{16})\,(7.0 \times 10^{4})\,(5.0 \times 10^{-5})^{2}(1.0 \times 10^{-4})^{2}}{(1.0 \times 10^{4})^{2}(0.10)^{2} + (2.5 \times 10^{16})\,(5.0 \times 10^{-5})^{2}(1.0 \times 10^{-4})^{2}}$$

or a D of 0.0438. The fraction of metal ion that remains in the aqueous phase is

$$(Q_{aq})_1 = \frac{100.0\ \text{mL}}{(0.0438)(10.00\ \text{mL}) + 100.0\ \text{mL}} = 0.996$$

At a pH of 1.00, we extract only 0.40% of the metal into the organic phase. Changing the pH to 3.00, however, increases the extraction efficiency to 97.8%. Figure 7.7.6 shows how the pH of the aqueous phase affects the extraction efficiency for M^{2+}.

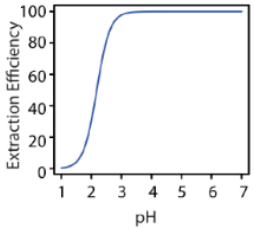


Figure 7.7.6 . Plot of extraction efficiency versus pH for the extraction of the metal ion, M^{2+}, in Example 7.7.4 .

One advantage of using a ligand to extract a metal ion is the high degree of selectivity that it brings to a liquid–liquid extraction. As seen in Figure 7.7.6 , a divalent metal ion's extraction efficiency increases from approximately 0% to 100% over a range of 2 pH units. Because a ligand's ability to form a metal–ligand complex varies substantially from metal ion to metal ion, significant selectivity is possible if we carefully control the pH. Table 7.7.1 shows the minimum pH for extracting 99% of a metal ion from an aqueous solution using an equal volume of 4 mM dithizone in CCl_4.

Table 7.7.1 . Minimum pH for Extracting 99% of an Aqueous Metal Ion Using 4.0 mM Dithizone in CCl_4 $V_{aq} = V_{org}$

metal ion	minimum pH
Hg^{2+}	–8.7
Ag^{+}	–1.7
Cu^{2+}	–0.8
Bi^{3+}	0.9
Zn^{2+}	2.3
Cd^{2+}	3.6
Co^{2+}	3.6
Pb^{2+}	4.1
Ni^{2+}	6.0
Tl^{+}	8.7

Example 7.7.5

Using Table 7.7.1 , explain how we can separate the metal ions in an aqueous mixture of Cu^{2+}, Cd^{2+}, and Ni^{2+} by extracting with an equal volume of dithizone in CCl_4.

Solution

From Table 7.7.1 , a quantitative separation of Cu^{2+} from Cd^{2+} and from Ni^{2+} is possible if we acidify the aqueous phase to a pH of less than 1. This pH is greater than the minimum pH for extracting Cu^{2+} and significantly less than the minimum pH for extracting either Cd^{2+} or Ni^{2+}. After the extraction of Cu^{2+} is complete, we shift the pH of the aqueous phase to 4.0, which allows us to extract Cd^{2+} while leaving Ni^{2+} in the aqueous phase.