

CHEMISTRY 2305



British Columbia Institute of Technology

Chem 2305

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CHAPTER OVERVIEW

1.1: An Introduction to Ultraviolet-Visible Absorption Spectrometry

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Thumbnail: Light dispersion of a mercury-vapor lamp with a flint glass prism/ Image used with permission (CC BY-SA 3.0; D-Kuru).

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1.2: Photoluminescent Spectroscopy

Photoluminescence is divided into two categories: fluorescence and phosphorescence. A pair of electrons that occupy the same electronic ground state have opposite spins and are in a singlet spin state (Figure 10.6.1 a).

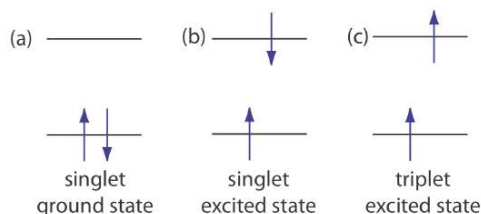


Figure 10.6.1 . Electron configurations for (a) a singlet ground state; (b) a singlet excited state; and (c) a triplet excited state.

When an analyte absorbs an ultraviolet or a visible photon, one of its valence electrons moves from the ground state to an excited state with a conservation of the electron's spin (Figure 10.6.1 b). Emission of a photon from a **singlet excited state** to the singlet ground state—or between any two energy levels with the same spin—is called **fluorescence**. The probability of fluorescence is very high and the average lifetime of an electron in the excited state is only 10^{-5} – 10^{-8} s. Fluorescence, therefore, rapidly decays once the source of excitation is removed.

In some cases an electron in a singlet excited state is transformed to a **triplet excited state** (Figure 10.6.1 c) in which its spin no is longer paired with the ground state. Emission between a triplet excited state and a singlet ground state—or between any two energy levels that differ in their respective spin states—is called **phosphorescence**. Because the average lifetime for phosphorescence ranges from 10^{-4} – 10^4 s, phosphorescence may continue for some time after we remove the excitation source.

The use of molecular fluorescence for qualitative analysis and for semi-quantitative analysis dates to the early to mid 1800s, with more accurate quantitative methods appearing in the 1920s. Instrumentation for fluorescence spectroscopy using a filter or a monochromator for wavelength selection appeared in, respectively, the 1930s and 1950s. Although the discovery of phosphorescence preceded that of fluorescence by almost 200 years, qualitative and quantitative applications of molecular phosphorescence did not receive much attention until after the development of fluorescence instrumentation.

As you might expect, the persistence of long-lived phosphorescence made it more noticeable.

Fluorescence and Phosphorescence Spectra

To appreciate the origin of fluorescence and phosphorescence we must consider what happens to a molecule following the absorption of a photon. Let's assume the molecule initially occupies the lowest vibrational energy level of its electronic ground state, which is the singlet state labeled S_0 in Figure 10.6.2 . Absorption of a photon excites the molecule to one of several vibrational energy levels in the first excited electronic state, S_1 , or the second electronic excited state, S_2 , both of which are singlet states. Relaxation to the ground state occurs by a number of mechanisms, some of which result in the emission of a photon and others that occur without the emission of a photon. These relaxation mechanisms are shown in Figure 10.6.2 . The most likely relaxation pathway from any excited state is the one with the shortest lifetime.

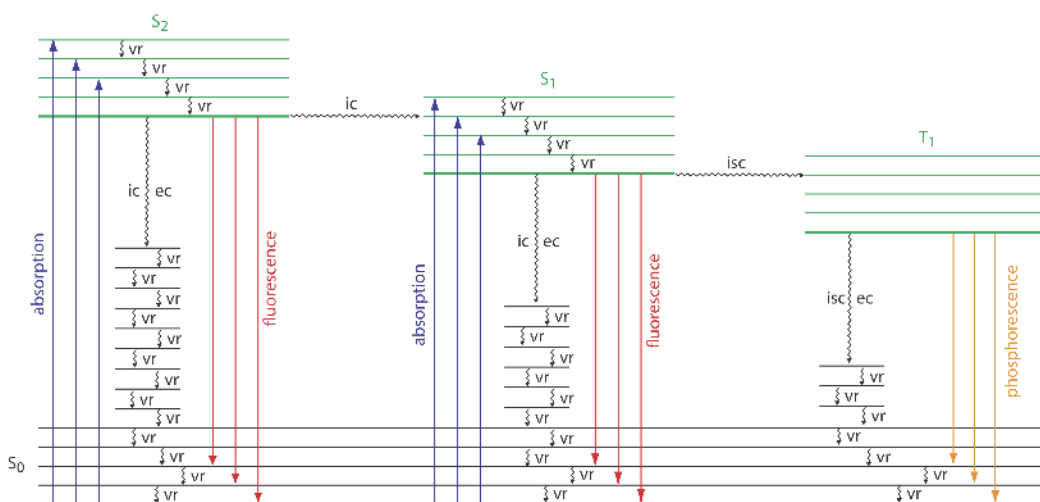


Figure 10.6.2 . Energy level diagram for a molecule that shows pathways for the deactivation of an excited state: **vr** is vibrational relaxation; **ic** is internal conversion; **ec** is external conversion; and **isc** is an intersystem crossing. The lowest vibrational energy for each electronic state is indicated by the thicker line. The electronic ground state is shown in black and the three electronic excited states are shown in green. The absorption, fluorescence, and phosphorescence of photons also are shown.

Radiationless Deactivation

When a molecule relaxes without emitting a photon we call the process **radiationless deactivation**. One example of radiationless deactivation is **vibrational relaxation**, in which a molecule in an excited vibrational energy level loses energy by moving to a lower vibrational energy level in the same electronic state. Vibrational relaxation is very rapid, with an average lifetime of $<10^{-12}$ s. Because vibrational relaxation is so efficient, a molecule in one of its excited state's higher vibrational energy levels quickly returns to the excited state's lowest vibrational energy level.

Another form of radiationless deactivation is an **internal conversion** in which a molecule in the ground vibrational level of an excited state passes directly into a higher vibrational energy level of a lower energy electronic state of the same spin state. By a combination of internal conversions and vibrational relaxations, a molecule in an excited electronic state may return to the ground electronic state without emitting a photon. A related form of radiationless deactivation is an **external conversion** in which excess energy is transferred to the solvent or to another component of the sample's matrix.

Let's use Figure 10.6.2 to illustrate how a molecule can relax back to its ground state without emitting a photon. Suppose our molecule is in the highest vibrational energy level of the second electronic excited state. After a series of vibrational relaxations brings the molecule to the lowest vibrational energy level of S_2 , it undergoes an internal conversion into a higher vibrational energy level of the first excited electronic state. Vibrational relaxations bring the molecule to the lowest vibrational energy level of S_1 . Following an internal conversion into a higher vibrational energy level of the ground state, the molecule continues to undergo vibrational relaxation until it reaches the lowest vibrational energy level of S_0 .

A final form of radiationless deactivation is an **intersystem crossing** in which a molecule in the ground vibrational energy level of an excited electronic state passes into one of the higher vibrational energy levels of a lower energy electronic state with a different spin state. For example, an intersystem crossing is shown in Figure 10.6.2 between the singlet excited state S_1 and the triplet excited state T_1 .

Relaxation by Fluorescence

Fluorescence occurs when a molecule in an excited state's lowest vibrational energy level returns to a lower energy electronic state by emitting a photon. Because molecules return to their ground state by the fastest mechanism, fluorescence is observed only if it is a more efficient means of relaxation than a combination of internal conversions and vibrational relaxations.

A quantitative expression of fluorescence efficiency is the fluorescent **quantum yield**, Φ_f , which is the fraction of excited state molecules that return to the ground state by fluorescence. The fluorescent quantum yields range from 1 when every molecule in an excited state undergoes fluorescence, to 0 when fluorescence does not occur.

The intensity of fluorescence, I_f , is proportional to the amount of radiation absorbed by the sample, $P_0 - P_T$, and the fluorescent quantum yield

$$I_f = k\Phi_f(P_0 - P_T) \quad (1.2.1)$$

where k is a constant that accounts for the efficiency of collecting and detecting the fluorescent emission. From Beer's law we know that

$$\frac{P_T}{P_0} = 10^{-\epsilon bC} \quad (1.2.2)$$

where C is the concentration of the fluorescing species. Solving Equation 1.2.2 for P_T and substituting into Equation 1.2.1 gives, after simplifying

$$I_f = k\Phi_f P_0 (1 - 10^{-\epsilon bC}) \quad (1.2.3)$$

When $\epsilon bC < 0.01$, which often is the case when the analyte's concentration is small, Equation 1.2.3 simplifies to

$$I_f = 2.303k\Phi_f \epsilon bC P_0 = k' P_0 \quad (1.2.4)$$

where k' is a collection of constants. The intensity of fluorescence, therefore, increases with an increase in the quantum efficiency, the source's incident power, and the molar absorptivity and the concentration of the fluorescing species.

Fluorescence generally is observed when the molecule's lowest energy absorption is a $\pi \rightarrow \pi^*$ transition, although some $n \rightarrow \pi^*$ transitions show weak fluorescence. Many unsubstituted, nonheterocyclic aromatic compounds have a favorable fluorescence quantum yield, although substitutions on the aromatic ring can effect Φ_f significantly. For example, the presence of an electron-withdrawing group, such as $-\text{NO}_2$, decreases Φ_f , while adding an electron-donating group, such as $-\text{OH}$, increases Φ_f . Fluorescence also increases for aromatic ring systems and for aromatic molecules with rigid planar structures. Figure 10.6.3 shows the fluorescence of quinine under a UV lamp.



Figure 10.6.3 . Tonic water, which contains quinine, is fluorescent when placed under a UV lamp. Source: Splarka (commons.Wikipedia.org).

A molecule's fluorescent quantum yield also is influenced by external variables, such as temperature and solvent. Increasing the temperature generally decreases Φ_f because more frequent collisions between the molecule and the solvent increases external conversion. A decrease in the solvent's viscosity decreases Φ_f for similar reasons. For an analyte with acidic or basic functional groups, a change in pH may change the analyte's structure and its fluorescent properties.

As shown in Figure 10.6.2 , fluorescence may return the molecule to any of several vibrational energy levels in the ground electronic state. Fluorescence, therefore, occurs over a range of wavelengths. Because the change in energy for fluorescent emission generally is less than that for absorption, a molecule's fluorescence spectrum is shifted to higher wavelengths than its absorption spectrum.

Relaxation by Phosphorescence

A molecule in a triplet electronic excited state's lowest vibrational energy level normally relaxes to the ground state by an intersystem crossing to a singlet state or by an external conversion. Phosphorescence occurs when the molecule relaxes by emitting a photon. As shown in Figure 10.6.2, phosphorescence occurs over a range of wavelengths, all of which are at lower energies than the molecule's absorption band. The intensity of phosphorescence, I_P , is given by an equation similar to Equation 1.2.4 for fluorescence

$$I_P = 2.303k\Phi_P\epsilon bCP_0 \quad (1.2.5)$$

$$= k'P_0 \quad (1.2.6)$$

where Φ_P is the **phosphorescent quantum yield**.

Phosphorescence is most favorable for molecules with $n \rightarrow \pi^*$ transitions, which have a higher probability for an intersystem crossing than $\pi \rightarrow \pi^*$ transitions. For example, phosphorescence is observed with aromatic molecules that contain carbonyl groups or heteroatoms. Aromatic compounds that contain halide atoms also have a higher efficiency for phosphorescence. In general, an increase in phosphorescence corresponds to a decrease in fluorescence.

Because the average lifetime for phosphorescence can be quite long, ranging from 10^{-4} – 10^4 s, the phosphorescent quantum yield usually is quite small. An improvement in Φ_P is realized by decreasing the efficiency of external conversion. This is accomplished in several ways, including lowering the temperature, using a more viscous solvent, depositing the sample on a solid substrate, or trapping the molecule in solution. Figure 10.6.4 shows an example of phosphorescence.

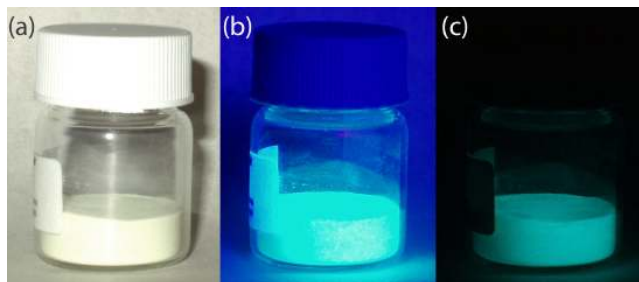


Figure 10.6.4. An europium doped strontium silicate-aluminum oxide powder under (a) natural light, (b) a long-wave UV lamp, and (c) in total darkness. The photo taken in total darkness shows the phosphorescent emission. Source: modified from Splarka ([commons.Wikipedia.org](https://commons.wikimedia.org/wiki/File:Eu-doped_SrSi2Al2O6.jpg)).

Excitation Versus Emission Spectra

Photoluminescence spectra are recorded by measuring the intensity of emitted radiation as a function of either the excitation wavelength or the emission wavelength. An **excitation spectrum** is obtained by monitoring emission at a fixed wavelength while varying the excitation wavelength. When corrected for variations in the source's intensity and the detector's response, a sample's excitation spectrum is nearly identical to its absorbance spectrum. The excitation spectrum provides a convenient means for selecting the best excitation wavelength for a quantitative or qualitative analysis.

In an **emission spectrum** a fixed wavelength is used to excite the sample and the intensity of emitted radiation is monitored as function of wavelength. Although a molecule has a single excitation spectrum, it has two emission spectra, one for fluorescence and one for phosphorescence. Figure 10.6.5 shows the UV absorption spectrum and the UV fluorescence emission spectrum for quinine.

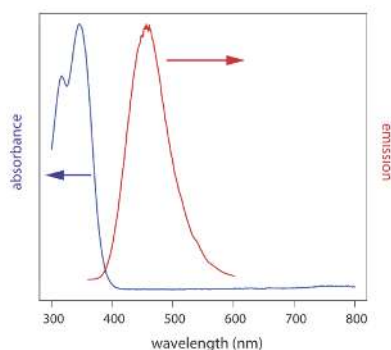


Figure 10.6.5 . Absorbance spectrum and fluorescence emission spectrum for quinine in 0.05 M H_2SO_4 . The emission spectrum uses an excitation wavelength of 350 nm with a bandwidth of 20 nm. Both spectra are normalized so that the maximum absorbance is 1.00 and the maximum emission is 1.00. The actual maximum absorbance is 0.444 and the actual maximum emission is 126747. Source: data from Daniel Scott, Department of Chemistry & Biochemistry, DePauw University.

Instrumentation

The basic instrumentation for monitoring fluorescence and phosphorescence—a source of radiation, a means of selecting a narrow band of radiation, and a detector—are the same as those for absorption spectroscopy. The unique demands of fluorescence and phosphorescence, however, require some modifications to the instrument designs seen earlier in Figure 10.3.1 (filter photometer), Figure 10.3.2 (single-beam spectrophotometer), Figure 10.3.3 (double-beam spectrophotometer), and Figure 10.3.4 (diode array spectrometer). The most important difference is that the detector cannot be placed directly across from the source. Figure 10.6.6 shows why this is the case. If we place the detector along the source's axis it receives both the transmitted source radiation, P_T , and the fluorescent, I_f , or phosphorescent, I_p , radiation. Instead, we rotate the detector and place it at 90° to the source.

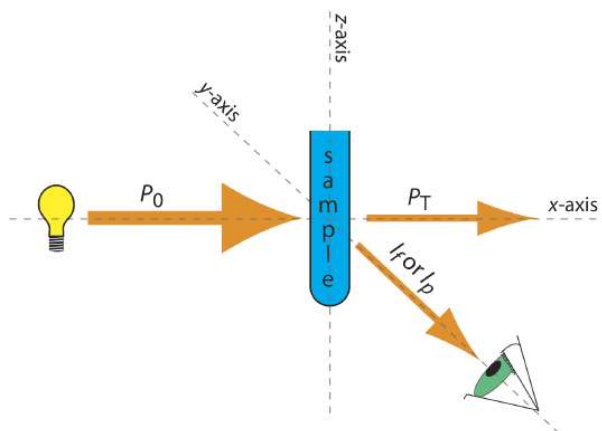


Figure 10.6.6 . Schematic diagram showing the orientation of the source and the detector when measuring fluorescence and phosphorescence. Contrast this to Figure 10.2.7, which shows the orientation for absorption spectroscopy.

Instruments for Measuring Fluorescence

Figure 10.6.7 shows the basic design of an instrument for measuring fluorescence, which includes two wavelength selectors, one for selecting the source's excitation wavelength and one for selecting the analyte's emission wavelength. In a **fluorimeter** the excitation and emission wavelengths are selected using absorption or interference filters. The excitation source for a fluorimeter usually is a low-pressure Hg vapor lamp that provides intense emission lines distributed throughout the ultraviolet and visible region. When a monochromator is used to select the excitation and the emission wavelengths, the instrument is called a **spectrofluorometer**. With a monochromator the excitation source usually is a high-pressure Xe arc lamp, which has a continuous emission spectrum. Either instrumental design is appropriate for quantitative work, although only a spectrofluorometer can record an excitation or emission spectrum.

A Hg vapor lamp has emission lines at 254, 312, 365, 405, 436, 546, 577, 691, and 773 nm.

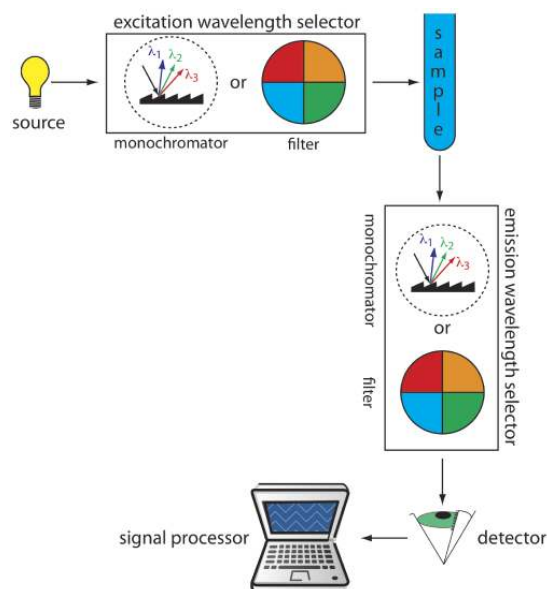


Figure 10.6.7 . Schematic diagram for measuring fluorescence showing the placement of the wavelength selectors for excitation and emission. When a filter is used the instrument is called a fluorimeter and when a monochromator is used the instrument is called a spectrofluorimeter.

The sample cells for molecular fluorescence are similar to those for molecular absorption (see Figure 10.3.6). Remote sensing using a fiber optic probe (see Figure 10.3.7) is possible using with either a fluorimeter or spectrofluorometer. An analyte that is fluorescent is monitored directly. For an analyte that is not fluorescent, a suitable fluorescent probe molecule is incorporated into the tip of the fiber optic probe. The analyte's reaction with the probe molecule leads to an increase or decrease in fluorescence.

Instruments for Measuring Phosphorescence

An instrument for molecular phosphorescence must discriminate between phosphorescence and fluorescence. Because the lifetime for fluorescence is shorter than that for phosphorescence, discrimination is achieved by incorporating a delay between exciting the sample and measuring the phosphorescent emission. Figure 10.6.8 shows how two out-of-phase choppers allow us to block fluorescent emission from reaching the detector when the sample is being excited and to prevent the source radiation from causing fluorescence when we are measuring the phosphorescent emission.

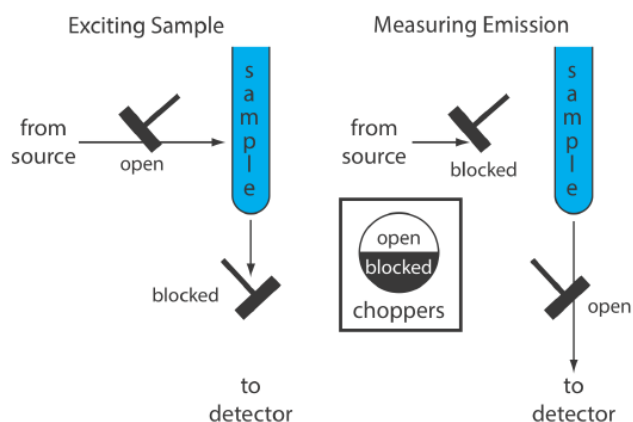


Figure 10.6.8 . Schematic diagram showing how choppers are used to prevent fluorescent emission from interfering with the measurement of phosphorescent emission.

Because phosphorescence is such a slow process, we must prevent the excited state from relaxing by external conversion. One way this is accomplished is by dissolving the sample in a suitable organic solvent, usually a mixture of ethanol, isopentane, and diethylether. The resulting solution is frozen at liquid-N₂ temperatures to form an optically clear solid. The solid matrix minimizes external conversion due to collisions between the analyte and the solvent. External conversion also is minimized by immobilizing the sample on a solid substrate, making possible room temperature measurements. One approach is to place a drop of a solution

that contains the analyte on a small disc of filter paper. After drying the sample under a heat lamp, the sample is placed in the spectrofluorometer for analysis. Other solid substrates include silica gel, alumina, sodium acetate, and sucrose. This approach is particularly useful for the analysis of thin layer chromatography plates.

Quantitative Applications

Molecular fluorescence and, to a lesser extent, phosphorescence are used for the direct or indirect quantitative analysis of analytes in a variety of matrices. A direct quantitative analysis is possible when the analyte's fluorescent or phosphorescent quantum yield is favorable. If the analyte is not fluorescent or phosphorescent, or if the quantum yield is unfavorable, then an indirect analysis may be feasible. One approach is to react the analyte with a reagent to form a product that is fluorescent or phosphorescent. Another approach is to measure a decrease in fluorescence or phosphorescence when the analyte is added to a solution that contains a fluorescent or phosphorescent probe molecule. A decrease in emission is observed when the reaction between the analyte and the probe molecule enhances radiationless deactivation or results in a nonemitting product. The application of fluorescence and phosphorescence to inorganic and organic analytes are considered in this section.

Inorganic Analytes

Except for a few metal ions, most notably UO_2^{2+} , most inorganic ions are not sufficiently fluorescent for a direct analysis. Many metal ions are determined indirectly by reacting with an organic ligand to form a fluorescent or, less commonly, a phosphorescent metal–ligand complex. One example is the reaction of Al^{3+} with the sodium salt of 2, 4, 3'-trihydroxyazobenzene-5'-sulfonic acid—also known as alizarin garnet R—which forms a fluorescent metal–ligand complex (Figure 10.6.9). The analysis is carried out using an excitation wavelength of 470 nm, with fluorescence monitored at 500 nm. Table 10.6.1 provides additional examples of chelating reagents that form fluorescent metal–ligand complexes with metal ions. A few inorganic nonmetals are determined by their ability to decrease, or quench, the fluorescence of another species. One example is the analysis for F^- based on its ability to quench the fluorescence of the Al^{3+} –alizarin garnet R complex.

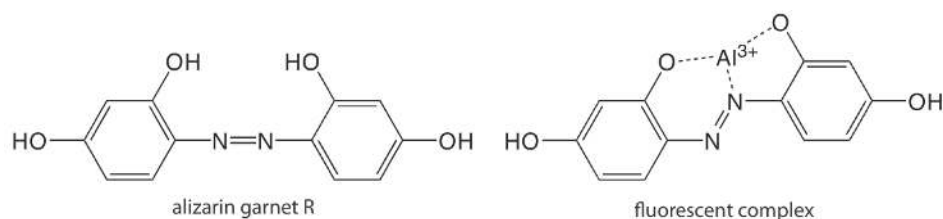


Figure 10.6.9 . Structure of alizarin garnet R and its metal–ligand complex with Al^{3+} .

Table 10.6.1 . Chelating Agents for the Fluorescent Analysis of Metal Ions

chelating agent	metal ions
8-hydroxyquinoline	Al^{3+} , Be^{2+} , Zn^{2+} , Li^+ , Mg^{2+} (and others)
flavonal	Zr^{2+} , Sn^{4+}
benzoin	$\text{B}_4\text{O}_6^{2-}$, Zn^{2+}
2', 3', 4', 5, 7 – pentahydroxyflavone	Be^{2+}
2-(o-hydroxyphenyl) benzoxazole	Cd^{2+}

Organic Analytes

As noted earlier, organic compounds that contain aromatic rings generally are fluorescent and aromatic heterocycles often are phosphorescent. Table 10.6.2 provides examples of several important biochemical, pharmaceutical, and environmental compounds that are analyzed quantitatively by fluorimetry or phosphorimetry. If an organic analyte is not naturally fluorescent or phosphorescent, it may be possible to incorporate it into a chemical reaction that produces a fluorescent or phosphorescent product. For example, the enzyme creatine phosphokinase is determined by using it to catalyze the formation of creatine from phosphocreatine. Reacting the creatine with ninhydrin produces a fluorescent product of unknown structure.

Table 10.6.2 . Examples of Naturally Photoluminescent Organic Analytes

class	compounds (F = fluorescence, P = phosphorescence)
aromatic amino acids	phenylalanine (F) tyrosine (F) tryptophan (F, P)
vitamins	vitamin A (F) vitamin B2 (F) vitamin B6 (F) vitamin B12 (F) vitamin E (F) folic acid (F)
catecholamines	dopamine (F) norepinephrine (F)
pharmaceuticals and drugs	quinine (F) salicylic acid (F, P) morphine (F) barbiturates (F) LSD (F) codeine (P) caffeine (P) sulfanilamide (P)
environmental pollutants	pyrene (F) benzo[a]pyrene (F) organothiophosphorous pesticides (F) carbamate insecticides (F) DDT (P)

Standardizing the Method

From Equation 1.2.4 and Equation 1.2.6 we know that the intensity of fluorescence or phosphorescence is a linear function of the analyte's concentration provided that the sample's absorbance of source radiation ($A = \epsilon bC$) is less than approximately 0.01. Calibration curves often are linear over four to six orders of magnitude for fluorescence and over two to four orders of magnitude for phosphorescence. For higher concentrations of analyte the calibration curve becomes nonlinear because the assumptions that led to Equation 1.2.4 and Equation 1.2.6 no longer apply. Nonlinearity may be observed for smaller concentrations of analyte fluorescent or phosphorescent contaminants are present. As discussed earlier, quantum efficiency is sensitive to temperature and sample matrix, both of which must be controlled when using external standards. In addition, emission intensity depends on the molar absorptivity of the photoluminescent species, which is sensitive to the sample matrix.

Representative Method 10.6.1: Determination of Quinine in Urine

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of quinine in urine provides an instructive example of a typical procedure. The description here is based on Mule, S. J.; Hushin, P. L. *Anal. Chem.* **1971**, 43, 708–711, and O'Reilly, J. E.; *J. Chem. Educ.* **1975**, 52, 610–612. Figure 10.6.3 shows the fluorescence of the quinine in tonic water.

Description of the Method

Quinine is an alkaloid used to treat malaria. It is a strongly fluorescent compound in dilute solutions of H_2SO_4 ($\Phi_f = 0.55$). Quinine's excitation spectrum has absorption bands at 250 nm and 350 nm and its emission spectrum has a single emission band at 450 nm. Quinine is excreted rapidly from the body in urine and is determined by measuring its fluorescence following its extraction from the urine sample.

Procedure

Transfer a 2.00-mL sample of urine to a 15-mL test tube and use 3.7 M NaOH to adjust its pH to between 9 and 10. Add 4 mL of a 3:1 (v/v) mixture of chloroform and isopropanol and shake the contents of the test tube for one minute. Allow the organic and the aqueous (urine) layers to separate and transfer the organic phase to a clean test tube. Add 2.00 mL of 0.05 M H_2SO_4 to the organic phase and shake the contents for one minute. Allow the organic and the aqueous layers to separate and transfer the aqueous phase to the sample cell. Measure the fluorescent emission at 450 nm using an excitation wavelength of 350 nm. Determine the concentration of quinine in the urine sample using a set of external standards in 0.05 M H_2SO_4 , prepared from a 100.0 ppm solution of quinine in 0.05 M H_2SO_4 . Use distilled water as a blank.

Questions

1. Chloride ion quenches the intensity of quinine's fluorescent emission. For example, in the presence of 100 ppm NaCl (61 ppm Cl^-) quinine's emission intensity is only 83% of its emission intensity in the absence of chloride. The presence of 1000 ppm NaCl (610 ppm Cl^-) further reduces quinine's fluorescent emission to less than 30% of its emission intensity in the absence of chloride. The concentration of chloride in urine typically ranges from 4600–6700 ppm Cl^- . Explain how this procedure prevents an interference from chloride.

The procedure uses two extractions. In the first of these extractions, quinine is separated from urine by extracting it into a mixture of chloroform and isopropanol, leaving the chloride ion behind in the original sample.

2. Samples of urine may contain small amounts of other fluorescent compounds, which will interfere with the analysis if they are carried through the two extractions. Explain how you can modify the procedure to take this into account?

One approach is to prepare a blank that uses a sample of urine known to be free of quinine. Subtracting the blank's fluorescent signal from the measured fluorescence from urine samples corrects for the interfering compounds.

The fluorescent emission for quinine at 450 nm can be induced using an excitation frequency of either 250 nm or 350 nm. The fluorescent quantum efficiency is the same for either excitation wavelength. Quinine's absorption spectrum shows that ϵ_{250} is greater than ϵ_{350} . Given that quinine has a stronger absorbance at 250 nm, explain why its fluorescent emission intensity is greater when using 350 nm as the excitation wavelength.

From Equation 1.2.4 we know that I_f is a function of the following terms: k , Φ_f , P_0 , ϵ , b , and C . We know that Φ_f , b , and C are the same for both excitation wavelengths and that ϵ is larger for a wavelength of 250 nm; we can, therefore, ignore these terms. The greater emission intensity when using an excitation wavelength of 350 nm must be due to a larger value for P_0 or k . In fact, P_0 at 350 nm for a high-pressure Xe arc lamp is about 170% of that at 250 nm. In addition, the sensitivity of a typical photomultiplier detector (which contributes to the value of k) at 350 nm is about 140% of that at 250 nm.

✓ Example 10.6.1

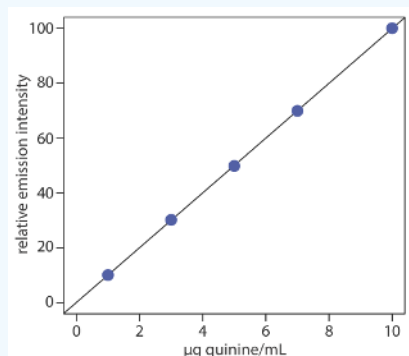
To evaluate the method described in Representative Method 10.6.1, a series of external standard are prepared and analyzed, providing the results shown in the following table. All fluorescent intensities are corrected using a blank prepared from a quinine-free sample of urine. The fluorescent intensities are normalized by setting I_f for the highest concentration standard to 100.

[quinine] ($\mu\text{g/mL}$)	I_f
1.00	10.11
3.00	30.20
5.00	49.84
7.00	69.89
10.00	100.0

After ingesting 10.0 mg of quinine, a volunteer provides a urine sample 24-h later. Analysis of the urine sample gives a relative emission intensity of 28.16. Report the concentration of quinine in the sample in mg/L and the percent recovery for the ingested quinine.

Solution

Linear regression of the relative emission intensity versus the concentration of quinine in the standards gives the calibration curve shown below and the following calibration equation.



$$I_f = 0.122 + 9.978 \times \frac{\text{g quinine}}{\text{mL}}$$

Substituting the sample's relative emission intensity into the calibration equation gives the concentration of quinine as 2.81 µg/mL. Because the volume of urine taken, 2.00 mL, is the same as the volume of 0.05 M H₂SO₄ used to extract the quinine, the concentration of quinine in the urine also is 2.81 µg/mL. The recovery of the ingested quinine is

$$\frac{\frac{2.81 \text{ µg quinine}}{\text{mL urine}} \times 2.00 \text{ mL urine} \times \frac{1 \text{ mg}}{1000 \text{ µg}}}{10.0 \text{ mg quinine ingested}} \times 100 = 0.0562\%$$

It can take 10–11 days for the body to completely excrete quinine so it is not surprising that such a small amount of quinine is recovered from this sample of urine.

Evaluation of Photoluminescence Spectroscopy

Scale of Operation

Photoluminescence spectroscopy is used for the routine analysis of trace and ultratrace analytes in macro and meso samples. Detection limits for fluorescence spectroscopy are influenced by the analyte's quantum yield. For an analyte with $\Phi_f > 0.5$, a picomolar detection limit is possible when using a high quality spectrofluorometer. For example, the detection limit for quinine sulfate, for which Φ is 0.55, generally is between 1 part per billion and 1 part per trillion. Detection limits for phosphorescence are somewhat higher, with typical values in the nanomolar range for low-temperature phosphorimetry and in the micromolar range for room-temperature phosphorimetry using a solid substrate.

Accuracy

The accuracy of a fluorescence method generally is between 1–5% when spectral and chemical interferences are insignificant. Accuracy is limited by the same types of problems that affect other optical spectroscopic methods. In addition, accuracy is affected by interferences that affect the fluorescent quantum yield. The accuracy of phosphorescence is somewhat greater than that for fluorescence.

Precision

The relative standard deviation for fluorescence usually is between 0.5–2% when the analyte's concentration is well above its detection limit. Precision usually is limited by the stability of the excitation source. The precision for phosphorescence often is limited by reproducibility in preparing samples for analysis, with relative standard deviations of 5–10% being common.

Sensitivity

From Equation 1.2.4 and Equation 1.2.6 we know that the sensitivity of a fluorescent or a phosphorescent method is affected by a number of parameters. We already have considered the importance of quantum yield and the effect of temperature and solution composition on Φ_f and Φ_p . Besides quantum yield, sensitivity is improved by using an excitation source that has a greater emission

intensity, P_0 , at the desired wavelength, and by selecting an excitation wavelength for which the analyte has a greater molar absorptivity, ϵ . Another approach for improving sensitivity is to increase the volume from which emission is monitored. Figure 10.6.10 shows how rotating a monochromator's slits from their usual vertical orientation to a horizontal orientation increases the sampling volume. The result can increase the emission from the sample by 5 – 30 $\times$.

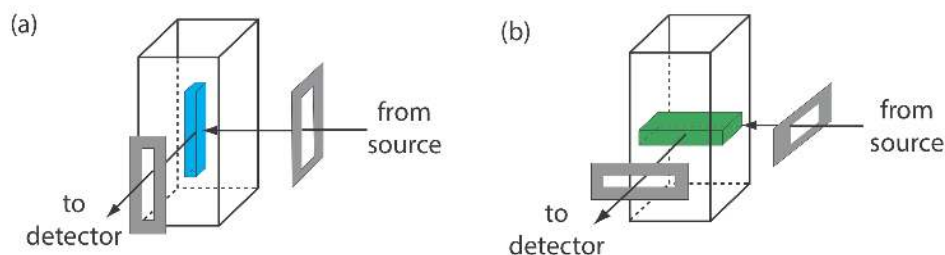


Figure 10.6.10 . Use of slit orientation to change the volume from which fluorescence is measured: (a) vertical slit orientation; (b) horizontal slit orientation. Suppose the slit's dimensions are 0.1 mm $\times$ 3 mm. In (a) the dimensions of the sampling volume are 0.1 mm $\times$ 0.1 mm $\times$ 3 mm, or 0.03 mm³. For (b) the dimensions of the sampling volume are 0.1 mm $\times$ 3 mm $\times$ 3 mm, or 0.9 mm³, a 30-fold increase in the sampling volume.

Selectivity

The selectivity of fluorescence and phosphorescence is superior to that of absorption spectrophotometry for two reasons: first, not every compound that absorbs radiation is fluorescent or phosphorescent; and, second, selectivity between an analyte and an interferent is possible if there is a difference in either their excitation or their emission spectra. The total emission intensity is a linear sum of that from each fluorescent or phosphorescent species. The analysis of a sample that contains n analytes, therefore, is accomplished by measuring the total emission intensity at n wavelengths.

Time, Cost, and Equipment

As with other optical spectroscopic methods, fluorescent and phosphorescent methods provide a rapid means for analyzing samples and are capable of automation. Fluorimeters are relatively inexpensive, ranging from several hundred to several thousand dollars, and often are satisfactory for quantitative work. Spectrofluorometers are more expensive, with models often exceeding \$50,000.

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CHAPTER OVERVIEW

1.2.1: Molecular Luminescence Spectrometry

Luminescence is emission of light by a substance not resulting from heat; it is thus a form of cold-body radiation. It can be caused by chemical reactions, electrical energy, subatomic motions, or stress on a crystal, which all are ultimately caused by Spontaneous emission. This distinguishes luminescence from incandescence, which is light emitted by a substance as a result of heating.

[1.2.1.1: Theory of Fluorescence and Phosphorescence](#)

[1.2.1.2: Fluorescence and Phosphorescence Instrumentation](#)

[1.2.1.3: Applications of Photoluminescence Methods](#)

[1.2.1.4: Chemiluminescence](#)

Thumbnail: Chemiluminescence after a reaction of hydrogen peroxide and luminol. This is an image from video https://youtu.be/8_82cNtZSQE. Image used with permission (CC BY-SA 4.0; [Tavo Romann](#)).

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1.2.1.3: Applications of Photoluminescence Methods

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SECTION OVERVIEW

1.2.1.4: Chemiluminescence

The aim of the book is to give a comprehensive account of the chemiluminescence subject suitable for students, research workers, professional analytical chemists and life scientists. The chemistry of each reagent that has been used in chemiluminescence is explained and the techniques for increasing the magnitude of the emitted signal are discussed. Further sections describe the different instrumental methods that have been used and examine the sort of work that has been carried out with them. The final sections discuss the applications of chemiluminescence in some of the major areas of analytical chemistry.

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1.3: Spectroscopy Based on Scattering

The blue color of the sky during the day and the red color of the sun at sunset are the result of light scattered by small particles of dust, molecules of water, and other gases in the atmosphere. The efficiency of a photon's scattering depends on its wavelength. We see the sky as blue during the day because violet and blue light scatter to a greater extent than other, longer wavelengths of light. For the same reason, the sun appears red at sunset because red light is less efficiently scattered and is more likely to pass through the atmosphere than other wavelengths of light. The scattering of radiation has been studied since the late 1800s, with applications beginning soon thereafter. The earliest quantitative applications of scattering, which date from the early 1900s, used the elastic scattering of light by colloidal suspensions to determine the concentration of colloidal particles.

Origin of Scattering

If we send a focused, monochromatic beam of radiation with a wavelength λ through a medium of particles with dimensions $< 1.5\lambda$, the radiation scatters in all directions. For example, visible radiation of 500 nm is scattered by particles as large as 750 nm in the longest dimension. Two general categories of scattering are recognized. In elastic scattering, radiation is first absorbed by the particles and then emitted without undergoing a change in the radiation's energy. When the radiation emerges with a change in energy, the scattering is inelastic. Only elastic scattering is considered in this text.

Elastic scattering is divided into two types: Rayleigh, or small-particle scattering, and large-particle scattering. Rayleigh scattering occurs when the scattering particle's largest dimension is less than 5% of the radiation's wavelength. The intensity of the scattered radiation is proportional to its frequency to the fourth power, ν^4 —which accounts for the greater scattering of blue light than red light—and is distributed symmetrically (Figure 10.8.1 a). For larger particles, scattering increases in the forward direction and decreases in the backward direction as the result of constructive and destructive interferences (Figure 10.8.1 b).

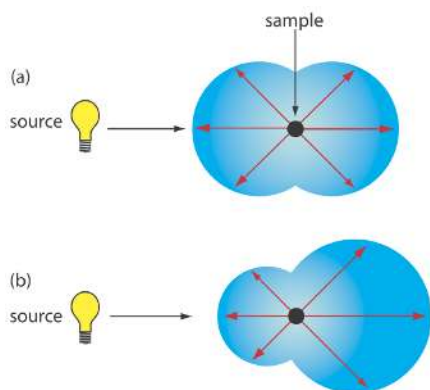


Figure 10.8.1 . Distribution of radiation for (a) Rayleigh, or small-particle scattering, and (b) large-particle scattering.

Turbidimetry and Nephelometry

Turbidimetry and nephelometry are two techniques that rely on the elastic scattering of radiation by a suspension of colloidal particles. In **turbidimetry** the detector is placed in line with the source and the decrease in the radiation's transmitted power is measured. In **nephelometry** the scattered radiation is measured at an angle of 90° to the source. The similarity of turbidimetry to absorbance spectroscopy and of nephelometry to fluorescence spectroscopy is evident in the instrumental designs shown in Figure 10.8.2 . In fact, we can use a UV/Vis spectrophotometer for turbidimetry and we can use a spectrofluorometer for nephelometry.

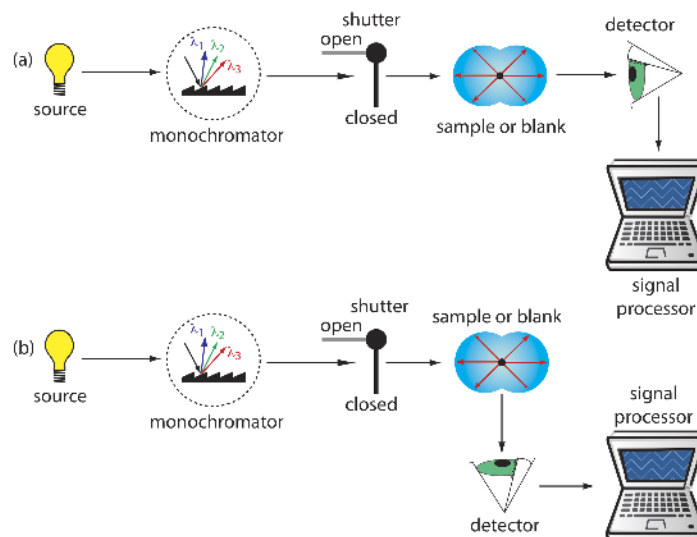


Figure 10.8.2 . Schematic diagrams for (a) a turbidimeter, and (b) a nephelometer.

Turbidimetry or Nephelometry?

When developing a scattering method the choice between using turbidimetry or using nephelometry is determined by two factors. The most important consideration is the intensity of the scattered radiation relative to the intensity of the source's radiation. If the solution contains a small concentration of scattering particles, then the intensity of the transmitted radiation, I_T , is approximately the same as the intensity of the source's radiation, I_0 . As we learned earlier in the section on molecular absorption, there is substantial uncertainty in determining a small difference between two intense signals. For this reason, nephelometry is a more appropriate choice for a sample that contains few scattering particles. Turbidimetry is a better choice when the sample contains a high concentration of scattering particles.

A second consideration in choosing between turbidimetry and nephelometry is the size of the scattering particles. For nephelometry, the intensity of scattered radiation at 90° increases when the particles are small and Rayleigh scattering is in effect. For larger particles, as shown in Figure 10.8.1 , the intensity of scattering decreases at 90° . When using an ultraviolet or a visible source of radiation, the optimum particle size is $0.1\text{--}1\text{ }\mu\text{m}$. The size of the scattering particles is less important for turbidimetry where the signal is the relative decrease in transmitted radiation. In fact, turbidimetric measurements are feasible even when the size of the scattering particles results in an increase in reflection and refraction, although a linear relationship between the signal and the concentration of scattering particles may no longer hold.

Determining Concentration by Turbidimetry

For turbidimetry the measured transmittance, T , is the ratio of the intensity of source radiation transmitted by the sample, I_T , to the intensity of source radiation transmitted by a blank, I_0 .

$$T = \frac{I_T}{I_0}$$

The relationship between transmittance and the concentration of the scattering particles is similar to that given by Beer's law

$$-\log T = kbC \quad (1.3.1)$$

where C is the concentration of the scattering particles in mass per unit volume (w/v), b is the pathlength, and k is a constant that depends on several factors, including the size and shape of the scattering particles and the wavelength of the source radiation. The exact relationship is established by a calibration curve prepared using a series of standards that contain known concentrations of analyte. As with Beer's law, Equation 1.3.1 may show appreciable deviations from linearity.

Determining Concentration by Nephelometry

For nephelometry the relationship between the intensity of scattered radiation, I_s , and the concentration of scattering particles is

$$I_s = kI_0C \quad (1.3.2)$$

where k is an empirical constant for the system and I_0 is the intensity of the source radiation. The value of k is determined from a calibration curve prepared using a series of standards that contain known concentrations of analyte.

Selecting a Wavelength for the Incident Radiation

The choice of wavelength is based primarily on the need to minimize potential interferences. For turbidimetry, where the incident radiation is transmitted through the sample, a monochromator or filter allow us to avoid wavelengths that are absorbed instead of scattered by the sample. For nephelometry, the absorption of incident radiation is not a problem unless it induces fluorescence from the sample. With a nonfluorescent sample there is no need for wavelength selection and a source of white light may be used as the incident radiation. For both techniques, other considerations in choosing a wavelength including the intensity of scattering, the transducer's sensitivity (many common photon transducers are more sensitive to radiation at 400 nm than at 600 nm), and the source's intensity.

Preparing the Sample for Analysis

Although Equation 1.3.1 and Equation 1.3.2 relate scattering to the concentration of the scattering particles, the intensity of scattered radiation also is influenced by the size and the shape of the scattering particles. Samples that contain the same number of scattering particles may show significantly different values for $-\log T$ or I_s depending on the average diameter of the particles. For a quantitative analysis, therefore, it is necessary to maintain a uniform distribution of particle sizes throughout the sample and between samples and standards.

Most turbidimetric and nephelometric methods rely on precipitation reaction to form the scattering particles. As we learned in Chapter 8, a precipitate's properties, including particle size, are determined by the conditions under which it forms. To maintain a reproducible distribution of particle sizes between samples and standards, it is necessary to control parameters such as the concentration of reagents, the order of adding reagents, the pH and temperature, the agitation or stirring rate, the ionic strength, and the time between the precipitate's initial formation and the measurement of transmittance or scattering. In many cases a surface-active agent—such as glycerol, gelatin, or dextrin—is added to stabilize the precipitate in a colloidal state and to prevent the coagulation of the particles.

Applications

Turbidimetry and nephelometry are used to determine the clarity of water. The primary standard for measuring clarity is formazin, an easily prepared, stable polymer suspension (Figure 10.8.3) [Hach, C. C.; Bryant, M. "Turbidity Standards," Technical Information Series, Booklet No. 12, Hach Company: Loveland, CO, 1995]. A stock standard of formazin is prepared by combining a 1g/100mL solution of hydrazine sulfate, $N_2H_4 \cdot H_2SO_4$, with a 10 g/100 mL solution of hexamethylenetetramine to produce a suspension of particles that is defined as 4000 nephelometric turbidity units (NTU). A set of external standards with NTUs between 0 and 40 is prepared by diluting the stock standard. This method is readily adapted to the analysis of the clarity of orange juice, beer, and maple syrup.

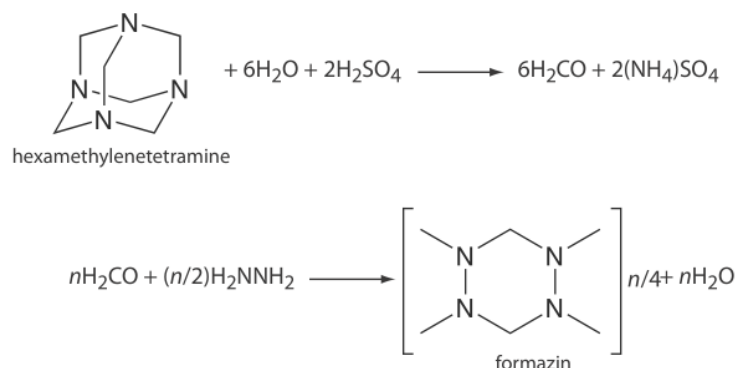


Figure 10.8.3 . Scheme for preparing formazin for use as a turbidity standard.

A number of inorganic cations and anions are determined by precipitating them under well-defined conditions. The transmittance or scattering of light, as defined by Equation 1.3.1 or Equation 1.3.2, is proportional to the concentration of the scattering particles, which, in turn, is related by the stoichiometry of the precipitation reaction to the analyte's concentration. Several examples of analytes determined in this way are listed in Table 10.8.1 .

Table 10.8.1 . Examples of Analytes Determined by Turbidimetry or Nephelometry

analyte	precipitant	precipitate
Ag^+	NaCl	AgCl
Ca^{2+}	$\text{Na}_2\text{C}_2\text{O}_4$	CaC_2O_4
Cl^-	AgNO_3	AgCl
CN^-	AgNO_3	AgCN
CO_3^{2-}	BaCl_2	BaCO_3
F^-	CaCl_2	CaF_2
SO_4^{2-}	BaCl_2	BaSO_4

Representative Method 10.8.1: Turbidimetric Determination of Sulfate in Water

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of sulfate in water provides an instructive example of a typical procedure. The description here is based on Method 4500-SO₄-C in *Standard Methods for the Analysis of Water and Wastewater*, American Public Health Association: Washington, D. C. 20th Ed., 1998.

Description of Method

Adding BaCl_2 to an acidified sample precipitates SO_4^{2-} as BaSO_4 . The concentration of SO_4^{2-} is determined either by turbidimetry or by nephelometry using an incident source of radiation of 420 nm. External standards that contain known concentrations of SO_4^{2-} are used to standardize the method.

Procedure

Transfer a 100-mL sample to a 250-mL Erlenmeyer flask along with 20.00 mL of an appropriate buffer. For a sample that contains more than 10 mg SO_4^{2-} /L, the buffer's composition is 30 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5 g of $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, 1.0 g of KNO_3 , and 20 mL of glacial CH_3COOH per liter. The buffer for a sample that contains less than 10 mg SO_4^{2-} /L is the same except for the addition of 0.111 g of Na_2SO_4 per L.

Place the sample and the buffer on a magnetic stirrer operated at the same speed for all samples and standards. Add a spoonful of 20–30 mesh BaCl_2 , using a measuring spoon with a capacity of 0.2–0.3 mL, to precipitate the SO_4^{2-} as BaSO_4 . Begin timing when the BaCl_2 is added and stir the suspension for 60 ± 2 s. When the stirring is complete, allow the solution to sit without stirring for 5.0 ± 0.5 min before measuring its transmittance or its scattering.

Prepare a calibration curve over the range 0–40 mg SO_4^{2-} /L by diluting a stock standard that is 100-mg SO_4^{2-} /L. Treat each standard using the procedure described above for the sample. Prepare a calibration curve and use it to determine the amount of sulfate in the sample.

Questions

1. What is the purpose of the buffer?

If the precipitate's particles are too small, I_T is too small to measure reliably. Because rapid precipitation favors the formation of micro-crystalline particles of BaSO_4 , we use conditions that favor the precipitate's growth over the nucleation of new particles. The buffer's high ionic strength and its acidity favor the precipitate's growth and prevent the formation of microcrystalline BaSO_4 .

2. Why is it important to use the same stirring rate and time for the samples and standards?

How fast and how long we stir the sample after we add BaCl_2 influences the size of the precipitate's particles.

3. Many natural waters have a slight color due to the presence of humic and fulvic acids, and may contain suspended matter (Figure 10.8.4). Explain why these might interfere with the analysis for sulfate. For each interferent, suggest a way to minimize its effect on the analysis.



Figure 10.8.4 . Waterfall on the River Swale in Richmond, England. The river, which flows out of the moors in the Yorkshire Dales, is brown in color as the result of organic matter that leaches from the peat found in the moors.

Suspended matter in a sample contributes to scattering and, therefore, results in a positive determinate error. We can eliminate this interference by filtering the sample prior to its analysis. A sample that is colored may absorb some of the source's radiation, leading to a positive determinate error. We can compensate for this interference by taking a sample through the analysis without adding BaCl_2 . Because no precipitate forms, we use the transmittance of this sample blank to correct for the interference.

4. Why is Na_2SO_4 added to the buffer for samples that contain less than $10 \text{ mg SO}_4^{2-}/\text{L}$?

The uncertainty in a calibration curve is smallest near its center. If a sample has a high concentration of SO_4^{2-} , we can dilute it so that its concentration falls near the middle of the calibration curve. For a sample with a small concentration of SO_4^{2-} , the buffer increases the concentration of sulfate by

$$\frac{0.111 \text{ g Na}_2\text{SO}_4}{\text{L}} \times \frac{96.06 \text{ g SO}_4^{2-}}{142.04 \text{ g Na}_2\text{SO}_4} \times \frac{1000 \text{ mg}}{\text{g}} \times \frac{20.00 \text{ mL}}{250.0 \text{ mL}} = 6.00 \text{ mg SO}_4^{2-}/\text{L}$$

After using the calibration curve to determine the amount of sulfate in the sample as analyzed, we subtract $6.00 \text{ mg SO}_4^{2-}/\text{L}$ to determine the amount of sulfate in the original sample.

✓ Example 10.8.1

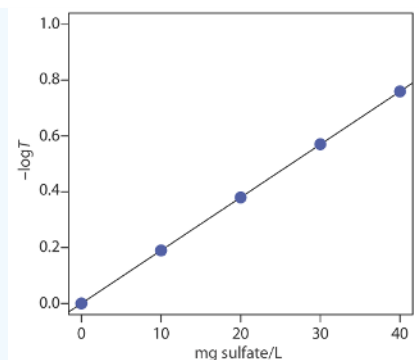
To evaluate the method described in Representative Method 10.8.1, a series of external standard was prepared and analyzed, providing the results shown in the following table.

mg $\text{SO}_4^{2-}/\text{L}$	transmittance
0.00	1.00
10.00	0.646
20.00	0.417
30.00	0.269
40.00	0.174

Analysis of a 100.0-mL sample of a surface water gives a transmittance of 0.538. What is the concentration of sulfate in the sample?

Solution

Linear regression of $-\log T$ versus concentration of SO_4^{2-} gives the calibration curve shown below, which has the following calibration equation.



$$-\log T = -1.04 \times 10^{-5} + 0.0190 \times \frac{\text{mg SO}_4^{2-}}{\text{L}}$$

Substituting the sample's transmittance into the calibration curve's equation gives the concentration of sulfate in sample as 14.2 mg SO_4^{2-} /L.

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CHAPTER OVERVIEW

1.4: An Introduction to Infrared Spectrometry

Infrared spectroscopy involves the interaction of infrared radiation with matter. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. Sample may be solid, liquid, or gas. The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far-infrared, named for their relation to the visible spectrum. The higher-energy near-IR, approximately $14000\text{--}4000\text{ cm}^{-1}$ ($0.8\text{--}2.5\text{ }\mu\text{m}$ wavelength) can excite overtone or harmonic vibrations. The mid-infrared, approximately $4000\text{--}400\text{ cm}^{-1}$ ($2.5\text{--}25\text{ }\mu\text{m}$) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The far-infrared, approximately $400\text{--}10\text{ cm}^{-1}$ ($25\text{--}1000\text{ }\mu\text{m}$), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The names and classifications of these subregions are conventions, and are only loosely based on the relative molecular or electromagnetic properties.

Topic hierarchy

[1.4.1: Theory of IR Absorption Spectrometry](#)

[1.4.2: 16.2 IR Instrumentation](#)

[1.4.3: IR Sources and Transducers](#)

Thumbnail: 3D animation of the symmetric stretching of the C–H bonds of bromomethane. Image used with permission (CC Y-SA 4.0; [Gaiabulbanix](#)).

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1.5: Atomic Emission Spectroscopy

The focus of this section is on the emission of ultraviolet and visible radiation following the thermal excitation of atoms. Atomic emission spectroscopy has a long history. Qualitative applications based on the color of flames were used in the smelting of ores as early as 1550 and were more fully developed around 1830 with the observation of atomic spectra generated by flame emission and spark emission [Dawson, J. B. *J. Anal. At. Spectrosc.* **1991**, 6, 93–98]. Quantitative applications based on the atomic emission from electric sparks were developed by Lockyer in the early 1870 and quantitative applications based on flame emission were pioneered by Lundegardh in 1930. Atomic emission based on emission from a plasma was introduced in 1964.

For an on-line introduction to much of the material in this section, see [Atomic Emission Spectroscopy \(AES\)](#) by Tomas Spudich and Alexander Scheeline, a resource that is part of the Analytical Sciences Digital Library.

Atomic Emission Spectra

Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy atomic orbital. Figure 10.7.1 shows a portion of the energy level diagram for sodium, which consists of a series of discrete lines at wavelengths that correspond to the difference in energy between two atomic orbitals.

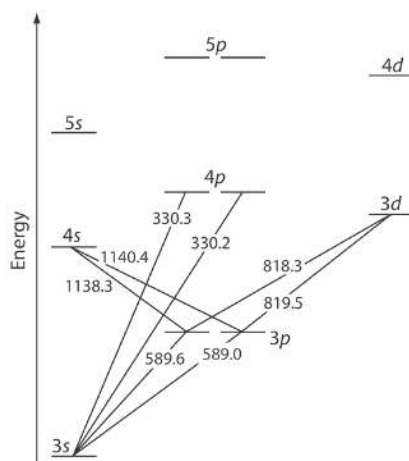


Figure 10.7.1 . Valence shell energy level diagram for sodium. The wavelengths corresponding to several transitions are shown. Note that this is the same energy level diagram as Figure 10.2.5.

The intensity of an atomic emission line, I_e , is proportional to the number of atoms, N^* , that populate the excited state,

$$I_e = kN^* \quad (1.5.1)$$

where k is a constant that accounts for the efficiency of the transition. If a system of atoms is in thermal equilibrium, the population of excited state i is related to the total concentration of atoms, N , by the Boltzmann distribution. For many elements at temperatures of less than 5000 K the Boltzmann distribution is approximated as

$$N^* = N \left(\frac{g_i}{g_0} \right) e^{-E_i/kT} \quad (1.5.2)$$

where g_i and g_0 are statistical factors that account for the number of equivalent energy levels for the excited state and the ground state, E_i is the energy of the excited state relative to a ground state energy, E_0 , k is Boltzmann's constant (1.3807×10^{-23} J/K), and T is the temperature in Kelvin. From Equation 1.5.2 we expect that excited states with lower energies have larger populations and more intense emission lines. We also expect emission intensity to increase with temperature.

Equipment

An atomic emission spectrometer is similar in design to the instrumentation for atomic absorption. In fact, it is easy to adapt most flame atomic absorption spectrometers for atomic emission by turning off the hollow cathode lamp and monitoring the difference between the emission intensity when aspirating the sample and when aspirating a blank. Many atomic emission spectrometers,

however, are dedicated instruments designed to take advantage of features unique to atomic emission, including the use of plasmas, arcs, sparks, and lasers as atomization and excitation sources, and an enhanced capability for multielemental analysis.

Atomization and Excitation

Atomic emission requires a means for converting into a free gaseous atom an analyte that is present in a solid, liquid, or solution sample. The same source of thermal energy used for atomization usually serves as the excitation source. The most common methods are flames and plasmas, both of which are useful for liquid or solution samples. Solid samples are analyzed by dissolving in a solvent and using a flame or plasma atomizer.

Flame Sources

Atomization and excitation in flame atomic emission is accomplished with the same nebulization and spray chamber assembly used in atomic absorption (Figure 10.4.1). The burner head consists of a single or multiple slots, or a Meker-style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

A Meker burner is similar to the more common Bunsen burner found in most laboratories; it is designed to allow for higher temperatures and for a larger diameter flame.

Plasma Sources

A **plasma** is a hot, partially ionized gas that contains an abundant concentration of cations and electrons. The plasma used in atomic emission is formed by ionizing a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating as the electrons and argon ions move through the gas. Because a plasma operates at a much higher temperature than a flame, it provides for a better atomization efficiency and a higher population of excited states.

A schematic diagram of the inductively coupled plasma source (ICP) is shown in Figure 10.7.2. The ICP torch consists of three concentric quartz tubes, surrounded at the top by a radio-frequency induction coil. The sample is mixed with a stream of Ar using a nebulizer, and is carried to the plasma through the torch's central capillary tube. Plasma formation is initiated by a spark from a Tesla coil. An alternating radio-frequency current in the induction coil creates a fluctuating magnetic field that induces the argon ions and the electrons to move in a circular path. The resulting collisions with the abundant unionized gas give rise to resistive heating, providing temperatures as high as 10000 K at the base of the plasma, and between 6000 and 8000 K at a height of 15–20 mm above the coil, where emission usually is measured. At these high temperatures the outer quartz tube must be thermally isolated from the plasma. This is accomplished by the tangential flow of argon shown in the schematic diagram.

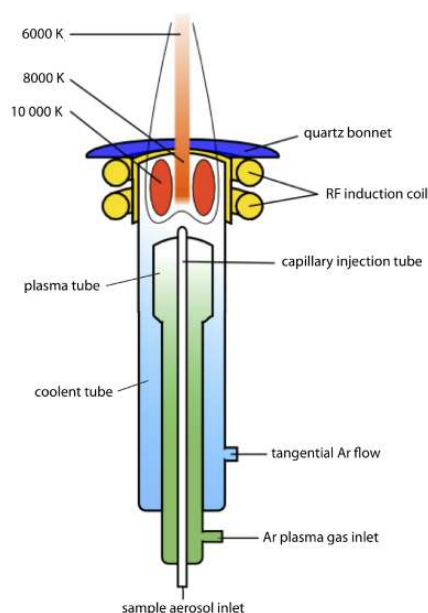


Figure 10.7.2 . Schematic diagram of an inductively coupled plasma torch.

Multielemental Analysis

Atomic emission spectroscopy is ideally suited for a multielemental analysis because all analytes in a sample are excited simultaneously. If the instrument includes a scanning monochromator, we can program it to move rapidly to an analyte's desired wavelength, pause to record its emission intensity, and then move to the next analyte's wavelength. This sequential analysis allows for a sampling rate of 3–4 analytes per minute.

Another approach to a multielemental analysis is to use a multichannel instrument that allows us to monitor simultaneously many analytes. A simple design for a multichannel spectrometer, shown in Figure 10.7.3, couples a monochromator with multiple detectors that are positioned in a semicircular array around the monochromator at positions that correspond to the wavelengths for the analytes.

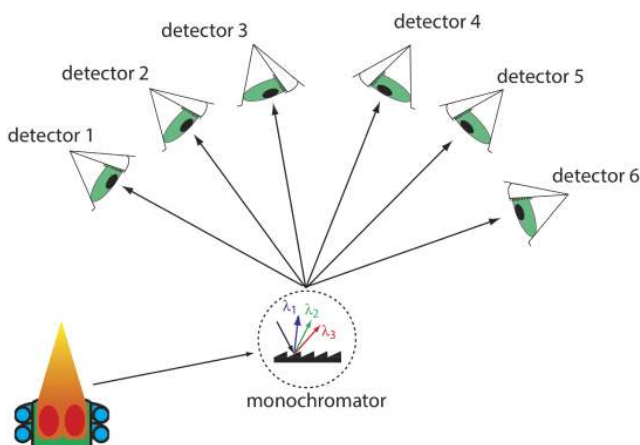


Figure 10.7.3 . Schematic diagram of a multichannel atomic emission spectrometer for the simultaneous analysis of several elements. Instruments may contain as many as 48–60 detectors.

Quantitative Applications

Atomic emission is used widely for the analysis of trace metals in a variety of sample matrices. The development of a quantitative atomic emission method requires several considerations, including choosing a source for atomization and excitation, selecting a wavelength and slit width, preparing the sample for analysis, minimizing spectral and chemical interferences, and selecting a method of standardization.

Choice of Atomization and Excitation Source

Except for the alkali metals, detection limits when using an ICP are significantly better than those obtained with flame emission (Table 10.7.1). Plasmas also are subject to fewer spectral and chemical interferences. For these reasons a plasma emission source is usually the better choice.

Table 10.7.1. Detection Limits for Atomic Emission

element	detection limit (µg/mL): flame emission	detection limit (µg/mL): ICP
Ag	2	0.2
Al	3	0.2
As	2000	2
Ca	0.1	0.0001
Cd	300	0.07
Co	5	0.1
Cr	1	0.08
Fe	10	0.09
Hg	150	1
K	0.01	30
Li	0.001	0.02
Mg	1	0.02
Mn	1	0.01
Na	0.01	0.1
Ni	10	0.2
Pb	0.2	1
Pt	2000	0.9
Sn	100	3
Zn	1000	0.1

Source: Parsons, M. L.; Major, S.; Forster, A. R.; App. Spectrosc. 1983, 37, 411–418.

Selecting the Wavelength and Slit Width

The choice of wavelength is dictated by the need for sensitivity and the need to avoid interferences from the emission lines of other constituents in the sample. Because an analyte's atomic emission spectrum has an abundance of emission lines—particularly when using a high temperature plasma source—it is inevitable that there will be some overlap between emission lines. For example, an analysis for Ni using the atomic emission line at 349.30 nm is complicated by the atomic emission line for Fe at 349.06 nm.

A narrower slit width provides better resolution, but at the cost of less radiation reaching the detector. The easiest approach to selecting a wavelength is to record the sample's emission spectrum and look for an emission line that provides an intense signal and is resolved from other emission lines.

Preparing the Sample

Flame and plasma sources are best suited for samples in solution and in liquid form. Although a solid sample can be analyzed by directly inserting it into the flame or plasma, they usually are first brought into solution by digestion or extraction.

Minimizing Spectral Interferences

The most important spectral interference is broad, background emission from the flame or plasma and emission bands from molecular species. This background emission is particularly severe for flames because the temperature is insufficient to break down refractory compounds, such as oxides and hydroxides. Background corrections for flame emission are made by scanning over the emission line and drawing a baseline (Figure 10.7.4). Because a plasma's temperature is much higher, a background interference

due to molecular emission is less of a problem. Although emission from the plasma's core is strong, it is insignificant at a height of 10–30 mm above the core where measurements normally are made.

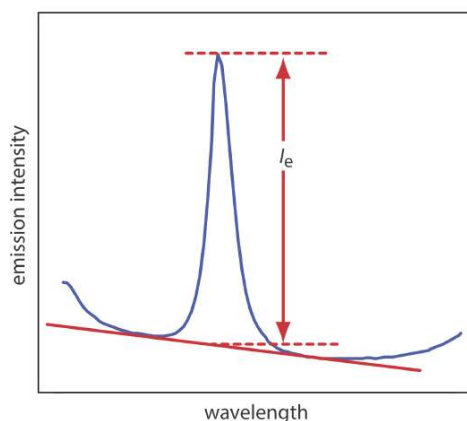


Figure 10.7.4 . Method for correcting an analyte's emission for the flame's background emission.

Minimizing Chemical Interferences

Flame emission is subject to the same types of chemical interferences as atomic absorption; they are minimized using the same methods: by adjusting the flame's composition and by adding protecting agents, releasing agents, or ionization suppressors. An additional chemical interference results from **self-absorption**. Because the flame's temperature is greatest at its center, the concentration of analyte atoms in an excited state is greater at the flame's center than at its outer edges. If an excited state atom in the flame's center emits a photon, then a ground state atom in the cooler, outer regions of the flame may absorb the photon, which decreases the emission intensity. For higher concentrations of analyte self-absorption may invert the center of the emission band (Figure 10.7.5).

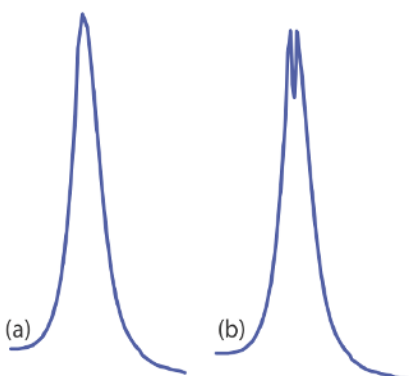


Figure 10.7.5 . Atomic emission lines for (a) a low concentration of analyte, and (b) a high concentration of analyte showing the effect of self-absorption.

Chemical interferences when using a plasma source generally are not significant because the plasma's higher temperature limits the formation of nonvolatile species. For example, PO_4^{3-} is a significant interferent when analyzing samples for Ca^{2+} by flame emission, but has a negligible effect when using a plasma source. In addition, the high concentration of electrons from the ionization of argon minimizes ionization interferences.

Standardizing the Method

From Equation 1.5.1 we know that emission intensity is proportional to the population of the analyte's excited state, N^* . If the flame or plasma is in thermal equilibrium, then the excited state population is proportional to the analyte's total population, N , through the Boltzmann distribution (Equation 1.5.2).

A calibration curve for flame emission usually is linear over two to three orders of magnitude, with ionization limiting linearity when the analyte's concentrations is small and self-absorption limiting linearity at higher concentrations of analyte. When using a

plasma, which suffers from fewer chemical interferences, the calibration curve often is linear over four to five orders of magnitude and is not affected significantly by changes in the matrix of the standards.

Emission intensity is affected significantly by many parameters, including the temperature of the excitation source and the efficiency of atomization. An increase in temperature of 10 K, for example, produces a 4% increase in the fraction of Na atoms in the 3p excited state, an uncertainty in the signal that may limit the use of external standards. The method of internal standards is used when the variations in source parameters are difficult to control. To compensate for changes in the temperature of the excitation source, the internal standard is selected so that its emission line is close to the analyte's emission line. In addition, the internal standard should be subject to the same chemical interferences to compensate for changes in atomization efficiency. To accurately correct for these errors the analyte and internal standard emission lines are monitored simultaneously.

Representative Method 10.7.1: Determination of Sodium in a Salt Substitute

The best way to appreciate the theoretical and the practical details discussed in this section is to carefully examine a typical analytical method. Although each method is unique, the following description of the determination of sodium in salt substitutes provides an instructive example of a typical procedure. The description here is based on Goodney, D. E. *J. Chem. Educ.* **1982**, 59, 875–876.

Description of Method

Salt substitutes, which are used in place of table salt for individuals on low-sodium diets, replaces NaCl with KCl. Depending on the brand, fumaric acid, calcium hydrogen phosphate, or potassium tartrate also are present. Although intended to be sodium-free, salt substitutes contain small amounts of NaCl as an impurity. Typically, the concentration of sodium in a salt substitute is about 100 µg/g. The exact concentration of sodium is determined by flame atomic emission. Because it is difficult to match the matrix of the standards to that of the sample, the analysis is accomplished by the method of standard additions.

Procedure

A sample is prepared by placing an approximately 10-g portion of the salt substitute in 10 mL of 3 M HCl and 100 mL of distilled water. After the sample has dissolved, it is transferred to a 250-mL volumetric flask and diluted to volume with distilled water. A series of standard additions is prepared by placing 25-mL portions of the diluted sample into separate 50-mL volumetric flasks, spiking each with a known amount of an approximately 10 mg/L standard solution of Na⁺, and diluting to volume. After zeroing the instrument with an appropriate blank, the instrument is optimized at a wavelength of 589.0 nm while aspirating a standard solution of Na⁺. The emission intensity is measured for each of the standard addition samples and the concentration of sodium in the salt substitute is reported in µg/g.

Questions

1. Potassium ionizes more easily than sodium. What problem might this present if you use external standards prepared from a stock solution of 10 mg Na/L instead of using a set of standard additions?

Because potassium is present at a much higher concentration than is sodium, its ionization suppresses the ionization of sodium. Normally suppressing ionization is a good thing because it increases emission intensity. In this case, however, the difference between the standard's matrix and the sample's matrix means that the sodium in a standard experiences more ionization than an equivalent amount of sodium in a sample. The result is a determinate error.

2. One way to avoid a determinate error when using external standards is to match the matrix of the standards to that of the sample. We could, for example, prepare external standards using reagent grade KCl to match the matrix to that of the sample. Why is this not a good idea for this analysis?

Sodium is a common contaminant in many chemicals. Reagent grade KCl, for example, may contain 40–50 µg Na/g. This is a significant source of sodium, given that the salt substitute contains approximately 100 µg Na/g.

3. Suppose you decide to use an external standardization. Given the previous questions, is the result of your analysis likely to underestimate or to overestimate the amount of sodium in the salt substitute?

The solid black line in Figure 10.7.6 shows the ideal calibration curve, assuming we match the standard's matrix to the sample's matrix, and that we do so without adding any additional sodium. If we prepare the external standards without adding KCl, the emission for each standard decreases due to increased ionization. This is shown by the lower of the two

dashed red lines. Preparing the standards by adding reagent grade KCl increases the concentration of sodium due to its contamination. Because we underestimate the actual concentration of sodium in the standards, the resulting calibration curve is shown by the other dashed red line. In both cases, the sample's emission results in our overestimating the concentration of sodium in the sample.

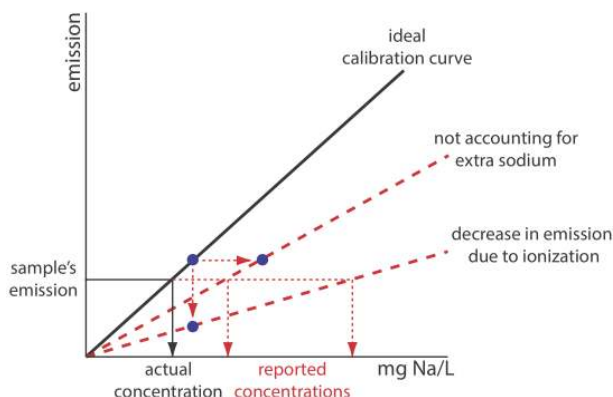


Figure 10.7.6 . External standards calibration curves for the flame atomic emission analysis of Na in a salt substitute. The solid black line shows the ideal calibration curve assuming we match the matrix of the samples and the standards using pure KCl. The lower of the two dashed red lines shows the effect of failing to add KCl to the external standards, which decreases emission. The other dashed red line shows the effect of using KCl that is contaminated with NaCl, which causes us to underestimate the concentration of Na in the standards. In both cases, the result is a positive determinate error in the analysis of samples.

4. One problem with analyzing salt samples is their tendency to clog the aspirator and burner assembly. What effect does this have on the analysis?

Clogging the aspirator and burner assembly decreases the rate of aspiration, which decreases the analyte's concentration in the flame. The result is a decrease in the emission intensity and a negative determinate error.

✓ Example 10.7.1

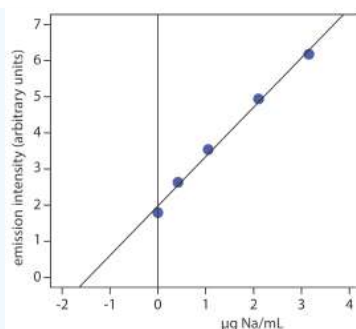
To evaluate the method described in Representative Method 10.7.1, a series of standard additions is prepared using a 10.0077-g sample of a salt substitute. The results of a flame atomic emission analysis of the standards is shown here [Goodney, D. E. *J. Chem. Educ.* **1982**, 59, 875–876].

added Na ($\mu\text{g/mL}$)	I_e (arb. units)
0.000	1.79
0.420	2.63
1.051	3.54
2.102	4.94
3.153	6.18

What is the concentration of sodium, in $\mu\text{g/g}$, in the salt substitute.

Solution

Linear regression of emission intensity versus the concentration of added Na gives the standard additions calibration curve shown below, which has the following calibration equation.



$$I_e = 1.97 + 1.37 \times \frac{\mu\text{g Na}}{\text{mL}}$$

The concentration of sodium in the sample is the absolute value of the calibration curve's x-intercept. Substituting zero for the emission intensity and solving for sodium's concentration gives a result of 1.44 µgNa/mL. The concentration of sodium in the salt substitute is

$$\frac{\frac{1.44 \mu\text{g Na}}{\text{mL}} \times \frac{50.00 \text{ mL}}{25.00 \text{ mL}} \times 250.0 \text{ mL}}{10.0077 \text{ g sample}} = 71.9 \mu\text{g Na/g}$$

Evaluation of Atomic Emission Spectroscopy

Scale of Operation

The scale of operations for atomic emission is ideal for the direct analysis of trace and ultratrace analytes in macro and meso samples. With appropriate dilutions, atomic emission can be applied to major and minor analytes.

Accuracy

When spectral and chemical interferences are insignificant, atomic emission can achieve quantitative results with accuracies of 1–5%. For flame emission, accuracy frequently is limited by chemical interferences. Because the higher temperature of a plasma source gives rise to more emission lines, accuracy when using plasma emission often is limited by stray radiation from overlapping emission lines.

Precision

For samples and standards in which the analyte's concentration exceeds the detection limit by at least a factor of 50, the relative standard deviation for both flame and plasma emission is about 1–5%. Perhaps the most important factor that affect precision is the stability of the flame's or the plasma's temperature. For example, in a 2500 K flame a temperature fluctuation of ± 2.5 K gives a relative standard deviation of 1% in emission intensity. Significant improvements in precision are realized when using internal standards.

Sensitivity

Sensitivity is influenced by the temperature of the excitation source and the composition of the sample matrix. Sensitivity is optimized by aspirating a standard solution of analyte and maximizing the emission by adjusting the flame's composition and the height from which we monitor the emission. Chemical interferences, when present, decrease the sensitivity of the analysis. Because the sensitivity of plasma emission is less affected by the sample matrix, a calibration curve prepared using standards in a matrix of distilled water is possible even for samples that have more complex matrices.

Selectivity

The selectivity of atomic emission is similar to that of atomic absorption. Atomic emission has the further advantage of rapid sequential or simultaneous analysis of multiple analytes.

Time, Cost, and Equipment

Sample throughput with atomic emission is rapid when using an automated system that can analyze multiple analytes. For example, sampling rates of 3000 determinations per hour are possible using a multichannel ICP, and sampling rates of 300 determinations per hour when using a sequential ICP. Flame emission often is accomplished using an atomic absorption spectrometer, which typically costs between \$10,000–\$50,000. Sequential ICP's range in price from \$55,000–\$150,000, while an ICP capable of simultaneous multielemental analysis costs between \$80,000–\$200,000. Combination ICP's that are capable of both sequential and simultaneous analysis range in price from \$150,000–\$300,000. The cost of Ar, which is consumed in significant quantities, can not be overlooked when considering the expense of operating an ICP.

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CHAPTER OVERVIEW

2.1: An Introduction to Chromatographic Separations

Topic hierarchy

- 2.1.1: General Description of Chromatography
- 2.1.2: Migration Rates of Solutes
- 2.1.3: Band Broadening and Column Efficiency
- 2.1.4: Optimization of Column Performance
- 2.1.5: Summary of Chromatographic Relationships
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Thumbnail: Separation of black ink on a thin layer chromatography plate. Image used with permission (CC BY-SA 3.0; [Natrij](#)).

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2.1.1: General Description of Chromatography

Chromatography refers to a group of methods that are used as a way of separating mixtures of compounds into their individual components. The basic set up of a chromatographic system is to have two phases, one stationary and one mobile. A compound, which we will usually call the solute, is introduced into the system, and essentially has a choice. If it is attracted to the mobile phase (has van der Waal attractions to the mobile phase), it will move through the system with the mobile phase. If it is attracted to the stationary phase, it will lag behind. It's easy to imagine some solute compounds with some degree of attraction for both phases, such that these move through the system with some intermediate rate of travel.

The first report of a chromatographic application was by Mikhail Tswett, a chemist from Estonia, in 1903. (A list of important literature articles is provided at the end). Undoubtedly other people had observed chromatography taking place, but no one until Tswett recognized its applicability for the separation of mixtures in chemistry. Tswett was interested in separating the pigments in plants. He packed a glass column that might have been comparable to a buret with starch, mashed up the plant and extracted the pigments into a solvent, loaded the solution onto the top of the starch column, and ran a mobile phase through the starch. Eventually he saw different color bands separate on the column, hence the term chromatography. Rather than eluting the colored bands off of the column, he stopped it, used a rod to push out the starch filling, divided up the bands of color, and extracted the individual pigments off the starch using an appropriate solvent. Tswett used a solid stationary phase (starch) and a liquid mobile phase.

Chromatographic systems can use either a gas or liquid as the mobile phase. Chromatographic systems can use either a solid or liquid as the stationary phase. A solid stationary phase is easy to imagine, as we have already seen for Tswett's work. Another common example is to use paper as the solid (actually, paper is made from a compound called cellulose). The sample is spotted onto the paper as shown in Figure 1a, and the bottom of the piece of paper is dipped into an appropriate liquid (note that the spot is above the liquid – Figure 1b). The liquid mobile phase moves up the paper by capillary action, and components of the mixture can separate into different spots depending on their relative attraction to the cellulose or chemicals that make up the liquid (Figure 1c).

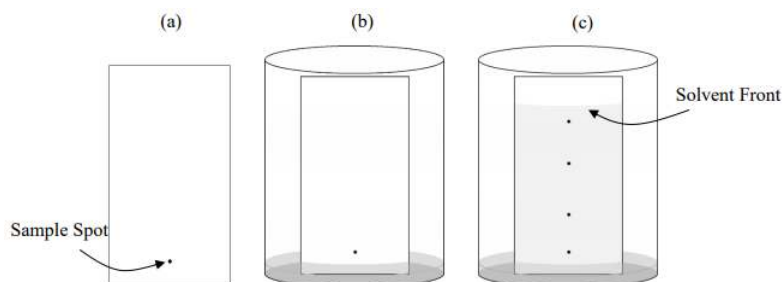


Figure 1. Paper chromatography

Another type of chromatography that you may be familiar with is thin layer chromatography (TLC). This is very similar to paper chromatography, although the stationary phase is usually a coating of small particles of a material known as silica gel (silica gel is a polymer with the formula SiO_2 , although a bit later we will talk in more detail about the exact chemical nature of this material) on a glass or plastic plate. Similarly, we could take these silica gel particles and pack them into a glass column, and then flow a liquid through the column. If we used some gas pressure to force the liquid through the silica gel column more quickly, we would have a common technique known as flash chromatography that synthetic chemists use to separate relatively large amounts of materials they have prepared. Another common solid to use in column chromatography is alumina (Al_2O_3 is the general formula, although we will say more later about its exact chemical nature).

These methods we have just been talking about are examples of liquid-solid chromatography. If we used gas as the mobile phase, injected either a gaseous sample or liquid sample into a high temperature zone that flash volatilized it, with some solid as the stationary phase (there are a range of possible materials we could use here), we could perform gas-solid chromatography. If we thought about how a solute compound would interact with such a solid stationary phase, we would realize that it must essentially “stick” to the surface by some intermolecular (van der Waal) forces. This sticking process in chromatography is known as **adsorption**.

Earlier I said that it is also possible to use a liquid as the stationary phase. This might seem odd at first, because it would seem as if the flowing mobile phase would somehow push along a liquid stationary phase. The way to use a liquid as the stationary phase is to coat a very thin layer of it onto a solid support, as illustrated in Figure 2.

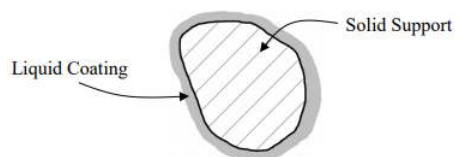


Figure 2. Liquid phase coated onto a solid support.

Alternatively, it is possible to coat a liquid phase onto the inside walls of a small-diameter, open-tubular column (known as a capillary column) as shown in Figure 3.



Figure 3. Liquid phase coated onto the inside walls of a capillary column.

The liquid coating has some attractive forces for the underlying solid surface, such that it sticks to the surface and stays in place. We could probably see how we could easily have a gas flowing around a packed bed of such particles and, so long as the liquid coating did not evaporate, it would stay in place on the particles. Flowing a liquid around it might be a bit more problematic, and we will examine that in length later in this unit. Suffice it to say, though, that it can be done. This would lead to gas-liquid and liquid-liquid chromatographic methods. The interaction of a solute compound with a liquid stationary phase is different than with the solid stationary phases discussed above. It will still depend on having attractive intermolecular forces, but instead of sticking to the solid surface by adsorption, the solute molecule will now dissolve into the liquid stationary phase. If a solute molecule dissolves into a liquid stationary phase in a chromatographic system, we say that the molecule **partitions** between the two phases.

These two terms are so important in chromatographic systems that it is worth summarizing them again:

Adsorption – describes the process of a solute molecule adhering to a solid surface

Partition – describes the process of a solute molecule dissolving into a liquid stationary phase

There are two terms we can describe in chromatography that are analogous to those we already described for liquid-liquid extraction. One of these is the **distribution constant (K_C)** that is the ratio of the concentration of the solute in the stationary phase (C_S) to that in the mobile phase (C_M).

$$K_C = \frac{C_S}{C_M} = \frac{\text{mol}_S / V_S}{\text{mol}_M / V_M} = \frac{\text{mol}_S \times V_M}{\text{mol}_M \times V_S} \quad (2.1.1.1)$$

But just like in liquid-liquid extraction, a problem with this ratio is that the volumes of the stationary and mobile phases might be significantly different from each other. With a solid stationary phase, V_S is comparable to the surface area of the particles. With a liquid stationary phase, the coating is usually so thin that V_M would be much larger than V_S . In many cases, a more useful term is the **partition coefficient (K_X)**, which is the ratio of the moles of solute in each of the two phases.

$$K_X = \frac{\text{mol}_S}{\text{mol}_M} \quad (2.1.1.2)$$

Notice that we can relate K_C to K_X because the mole ratio shows up in both. That allows us to write the following:

$$K_C = K_X \left(\frac{V_M}{V_S} \right) \quad (2.1.1.3)$$

There are also some other fundamental figures of merit that we often use when discussing chromatographic separations. The first is known as the **selectivity factor (α)**. In order to separate two components of a mixture, it is essential that the two have different distribution or partition coefficients (note, it does not matter which one you use since the volume terms will cancel if K_C is used).

The separation factor is the ratio of these two coefficients, and is always written so that it is greater than or equal to one. K_2 represents the distribution coefficient for the later eluting of the two components.

$$\alpha = \frac{K_2}{K_1} \quad (2.1.1.4)$$

If $\alpha = 1$, then there will be no separation of the two components. The larger the value of α , the greater the separation. One thing to realize is that there is some limit as to how large we want an α -value to be. For example, the chromatogram in Figure 4b has a much greater α value than that in Figure 4a. But the two components are fully separated so that the extra time we would need to wait for the chromatogram in Figure 4b is undesirable. In most chromatographic separations, the goal is to get just enough separation, so that we can keep the analysis time as short as possible.



Figure 4. Elution of compounds where the α -value in (b) is much greater than the α -value in (a).

Another important figure of merit in chromatographic separations is known as the **retention factor (k)**, which is a measure of the retention of a solute, or how much attraction the solute has for the stationary phase. The k term was formerly known as the capacity factor. The retention factor is actually identical to the partition coefficient.

$$k = \frac{\text{mol}_S}{\text{mol}_M} = \frac{C_S V_S}{C_M V_M} = K_C \left(\frac{V_S}{V_M} \right) \quad (2.1.1.5)$$

We can determine k values from a chromatogram using the following equation:

$$k = \frac{t_R - t_0}{t_0} \quad (2.1.1.6)$$

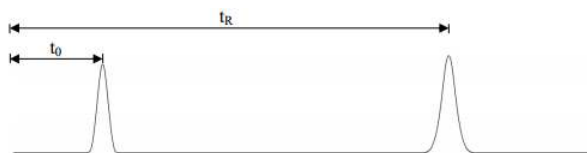


Figure 5. Chromatographic information used to calculate the k value for a peak.

As seen in Figure 5, the term t_R is the retention time of the compound of interest, whereas t_0 refers to the retention time of an unretained compound (the time it takes a compound with no ability to partition into or adsorb onto the stationary phase to move through the column). Since t_0 will vary from column to column, the form of this equation represents a normalization of the retention times to t_0 .

Another important figure of merit for a chromatographic column is known as the number of **theoretical plates (N)**. It turns out that one helpful way to think of a chromatographic column is as a series of microscopically thin plates as shown in Figure 6. Using the picture in Figure 6, we



Figure 6. Representation of a chromatographic column as a series of microscopically thin plates.

could imagine a compound moving down the column as a series of steps, one plate at a time. The compound enters a plate, distributes according to its distribution coefficient between the stationary and mobile phase, and then moves on to the next plate. Now it turns out that chromatographic systems never really reach equilibrium, and in fact are not steady state systems, but things like the distribution and partition coefficients, which are equilibrium expressions, are useful ways to examine the distribution in chromatographic systems. A compound with less favorable solubility in the stationary phase would then move through the series of plates faster. The only way to separate two compounds is to have enough plates, or enough equilibrations, to exploit the difference in partition coefficients between the two compounds. A column with more theoretical plates is more likely to separate two compounds than one with fewer.

We can determine the number of plates for a compound as shown in Figure 7.

$$N = 16 \left(\frac{t_R}{W} \right)^2 \quad (2.1.1.7)$$

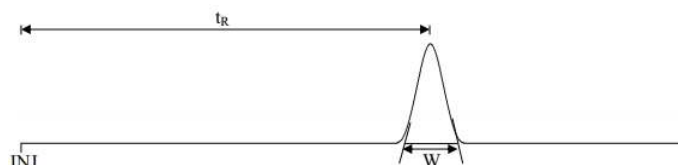


Figure 7. Chromatographic information used to determine N for a column.

W is the width of the peak where it intersects with the baseline. The important thing to remember is to use the same units when measuring W and t_R (e.g., distance in cm on a plot, time in seconds, elution volume in mL – which is common in liquid chromatography). It should be pointed out that a column will have a set number of plates that will not vary much from compound to compound. The reason for this is that a compound with a longer retention time will exhibit a larger peak width, such that the ratio term is correcting for these two effects.

If we determine the number of plates for a column, dividing the column length (L) by the number of plates leads to the **height equivalent to a theoretical plate (H)**. Note that the smaller the value of H the better. It actually turns out the H is the important figure of merit for a column.

$$H = \frac{L}{N} \quad (2.1.1.8)$$

The chromatograms in Figure 8 show the distinction between a column with a smaller value of H (Figure 8a) and one with a much larger value of H (Figure 8b).

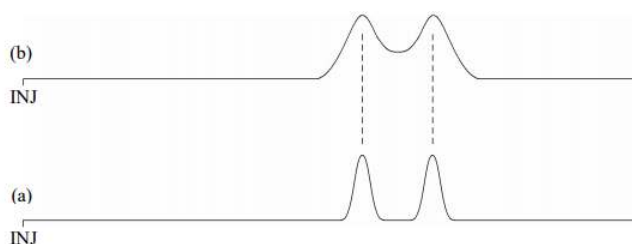


Figure 8. Chromatograms for a column with (a) a smaller value of H and (b) a larger value of H .

Usually today, though, people refer to something called the **reduced plate height (h)** for a column. The reduced plate height is defined as shown below, in which d_p is the particle diameter of the packing material used in the column (or the internal tube diameter if an open tubular capillary column is used).

$$h = \frac{H}{d_p} \quad (2.1.1.9)$$

You will also come across a term known as the **reduced velocity (ν)** in the chromatographic literature. The reduced velocity is defined as follows, in which v is the mobile phase velocity, and D_M the diffusion coefficient of the solute in the mobile phase.

$$\nu = \frac{d_p \times v}{D_M} \quad (2.1.1.10)$$

Finally, we can define an equation for the **resolution (R_s)** of two compounds. This will be a measure of how much two compounds in a chromatogram are separated from each other.

$$R_s = \frac{2(t_2 - t_1)}{W_1 + W_2} \quad (2.1.1.11)$$

The terms t_2 and t_1 refer to the retention time of the two compounds, and W_1 and W_2 to the width of each peak at baseline.

Figure 9 shows the separation of two peaks at resolution values of 0.75 (Figure 9a), 1.0 (Figure 9b) and 1.5 (Figure 9c). The better separation of A and B that occurs with increasing resolution is obvious. It is also worth noting that the analysis time is longer the higher the resolution. Chromatographic analyses are always a compromise between the degree of separation of the peaks and how long it takes to perform the analysis. In many cases, the goal is to get just enough separation in the shortest period of time.

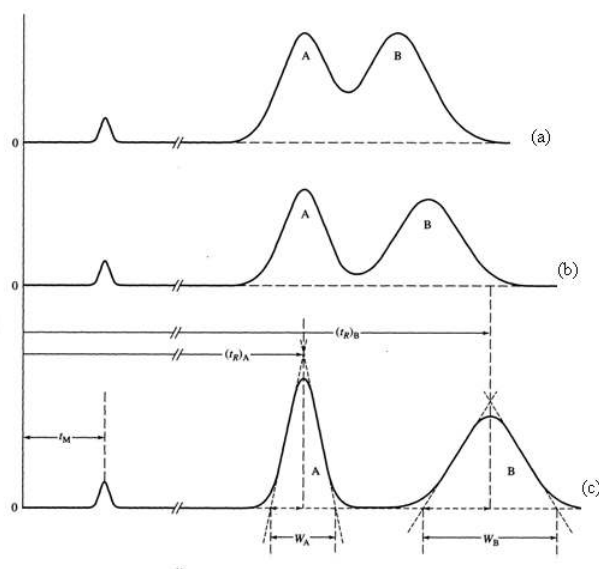


Figure 9. Separation of two peaks with resolution values of (a) 0.75, (b) 1.0 and (c) 1.5.

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SECTION OVERVIEW

2.1.3: Band Broadening and Column Efficiency

One of the most important occurrences in chromatographic systems is the broadening of peaks as compounds move through the chromatographic column. For example, if we examine Figure 18 in which both sets of peaks have the same retention times but different extents of broadening, we see that the set of conditions that produce the narrower set of peaks (Figure 18b) resulted in “better” or more efficient chromatography. What we observe in the chromatogram with less peak broadening is that the peaks are fully resolved and that we could fit more peaks into a similar window of time in the chromatogram. An ideal chromatographic system would therefore produce peaks that were straight line spikes in which no broadening occurred (Figure 18a).

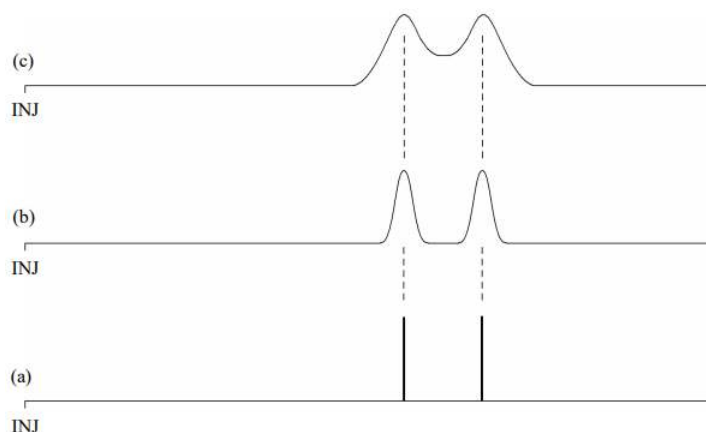


Figure 18. Representation of chromatographic peaks in which the broadening increases going from (a) to (c).

There are specific processes that occur in chromatographic systems that cause peaks to broaden. It also turns out that these processes are often influenced by experimental variables that we may have some control over. Contributions to broadening in chromatographic systems can be divided into two broad areas of concern. One is the contribution from what is known as **dead volume**. Dead volume refers to all the volume in a chromatographic system from the injector to the detector other than the column. Remember, the separation only occurs in the column. All other volumes (tubing used to connect components, volume within the detector cell, etc.) have the ability to contribute to peak broadening but not to the separation. One general goal then is to try to reduce the total dead volume to as small a quantity as possible. In liquid chromatography this involves using very narrow internal diameter tubing and short lengths of tubing to connect the components, using small-volume detector cells, etc. The fittings that are used to connect pieces of tubing together or components to each other have been designed specifically to reduce the dead volume to minimal levels and in ways that do not promote mixing and broadening.

The other source of broadening is within the column. If you were to examine state-of-the-art columns that are used today in gas and liquid chromatography, it turns out that there are several features of their design that lead to significant reductions in peak broadening. In other words, these columns represent the best we can do today to reduce the broadening of peaks, and therefore represent the most efficient column technology. It is worth taking the time to understand the various contributions to peak broadening that occur within the chromatographic column and to examine the ways in which current gas and liquid chromatographic columns have been designed to minimize these effects.

There are four general contributions to broadening within chromatographic columns. These are known as:

- longitudinal diffusion
- eddy diffusion
- mass transport broadening in the stationary phase
- mass transport broadening in the mobile phase

Before moving on, it is worth remembering back to two fundamental criteria we talked about with regards to chromatographic columns, the number of theoretical plates (N) and the reduced plate height (h). Remember that a column with more plates, or better

yet a column with a small reduced plate height, was more efficient and provided better separations. We can therefore use the reduced plate height as a determining measure of the efficiency of a chromatographic column. **The smaller the value of h , the more efficient the column.** What we will develop as we analyze the four contributions to broadening above is an equation, which was first known as the van Deemter equation (J. J. van Deemter described the first treatment of this for chromatographic systems in 1956), that relates these four terms to the reduced plate height.

Topic hierarchy

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SECTION OVERVIEW

2.1.4: Optimization of Column Performance

Another important equation in chromatographic separations is the fundamental resolution equation. The equation relates the resolution of two compounds (R_S) to the number of theoretical plates (N), retention or capacity factor (k), and selectivity factor (α). [Appendix I](#) shows the derivation of the fundamental resolution equation. Since we would usually be interested in separating two compounds that are close to each other in a chromatogram, and therefore possibly not resolved, we usually think of applying this equation when we want to improve the resolution of two adjacent components.

$$R_S = \left(\frac{\sqrt{N}}{4} \right) \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_2}{1 + k_2} \right) \quad (2.1.4.1)$$

The interesting part about this equation is that it is possible to examine a chromatogram with poor resolution, decide which of the three terms is causing the most serious problem, and then make systematic experimental changes to improve the results. It is worth examining the types of experimental changes that can be made to influence each of these terms. Notice that in each case, making the term larger will improve the resolution.

Topic hierarchy

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2.2: Gas Chromatography

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CHAPTER OVERVIEW

2.2.1: Gas Chromatography

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. [https://chem.libretexts.org/Under_Co...otography_\(GC\)](https://chem.libretexts.org/Under_Co...otography_(GC))

Topic hierarchy

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[2.2.1.2: Instruments for GLC](#)

[2.2.1.3: Gas Chromatographic Columns and Stationary Phases](#)

[2.2.1.4: Applications of GC](#)

[2.2.1.5: Advances in GC](#)

[2.2.1.6: Gas-Solid Chromatography](#)

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CHAPTER OVERVIEW

2.2.2: Molecular Mass Spectrometry

Mass spectrometry is an analytical technique that ionizes chemical species and sorts the ions based on their mass-to-charge ratio. In simpler terms, a mass spectrum measures the masses within a sample. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds.

Topic hierarchy

[2.2.2.1: Molecular Mass Spectra](#)

[2.2.2.2: Ion Sources](#)

[2.2.2.3: Mass Spectrometers](#)

[2.2.2.4: Applications of Molecular Mass Spectrometry](#)

[2.2.2.5: Quantitative Applications of Mass Spectrometry](#)

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2.2.2.3: Mass Spectrometers

This page describes how a mass spectrum is produced using a mass spectrometer.

How a mass spectrometer works

If something is moving and you subject it to a sideways force, instead of moving in a straight line, it will move in a curve - deflected out of its original path by the sideways force. Suppose you had a cannonball traveling past you and you wanted to deflect it as it went by you. All you've got is a jet of water from a hose-pipe that you can squirt at it. Frankly, it's not going to make a lot of difference! Because the cannonball is so heavy, it will hardly be deflected at all from its original course. But suppose instead, you tried to deflect a table tennis ball traveling at the same speed as the cannonball using the same jet of water. Because this ball is so light, you will get a huge deflection.

The amount of deflection you will get for a given sideways force depends on the mass of the ball. If you knew the speed of the ball and the size of the force, you could calculate the mass of the ball if you knew what sort of curved path it was deflected through. The less the deflection, the heavier the ball. You can apply exactly the same principle to atomic sized particles.

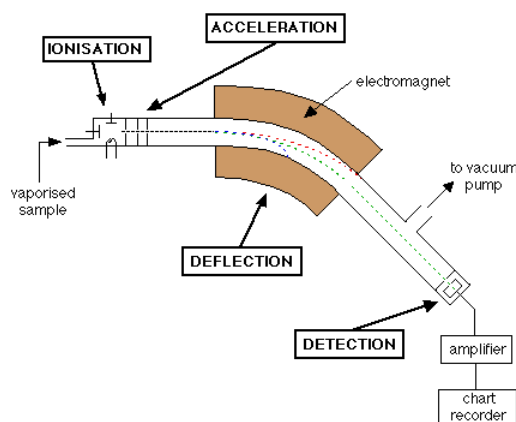
An outline of what happens in a mass spectrometer

Atoms can be deflected by magnetic fields - provided the atom is first turned into an ion. Electrically charged particles are affected by a magnetic field although electrically neutral ones aren't.

The sequence is :

- **Stage 1: Ionization:** The atom is ionised by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions.
- **Stage 2: Acceleration:** The ions are accelerated so that they all have the same kinetic energy.
- **Stage 3: Deflection:** The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected. The amount of deflection also depends on the number of positive charges on the ion - in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.
- **Stage 4: Detection:** The beam of ions passing through the machine is detected electrically.

A full diagram of a mass spectrometer



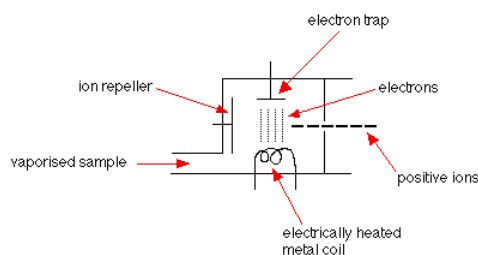
Understanding what's going on

The need for a vacuum

It's important that the ions produced in the ionization chamber have a free run through the machine without hitting air molecules.

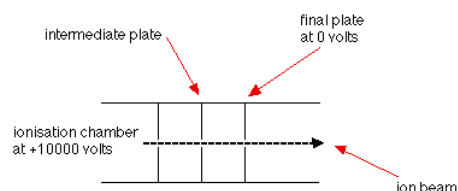
Ionization

The vaporized sample passes into the ionization chamber. The electrically heated metal coil gives off electrons which are attracted to the electron trap which is a positively charged plate.



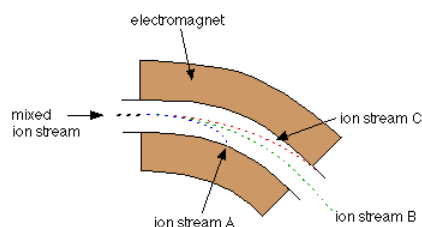
The particles in the sample (atoms or molecules) are therefore bombarded with a stream of electrons, and some of the collisions are energetic enough to knock one or more electrons out of the sample particles to make positive ions. Most of the positive ions formed will carry a charge of +1 because it is much more difficult to remove further electrons from an already positive ion. These positive ions are persuaded out into the rest of the machine by the ion repeller which is another metal plate carrying a slight positive charge.

Acceleration



The positive ions are repelled away from the very positive ionization chamber and pass through three slits, the final one of which is at 0 volts. The middle slit carries some intermediate voltage. All the ions are accelerated into a finely focused beam.

Deflection



Different ions are deflected by the magnetic field by different amounts. The amount of deflection depends on:

- the mass of the ion. Lighter ions are deflected more than heavier ones.
- the charge on the ion. Ions with 2 (or more) positive charges are deflected more than ones with only 1 positive charge.

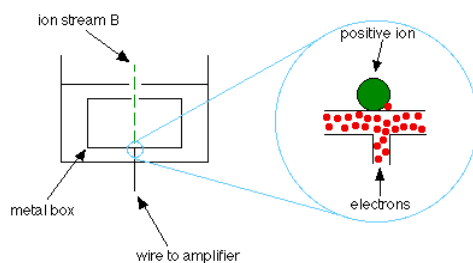
These two factors are combined into the mass/charge ratio. Mass/charge ratio is given the symbol m/z (or sometimes m/e). For example, if an ion had a mass of 28 and a charge of $1+$, its mass/charge ratio would be 28. An ion with a mass of 56 and a charge of $2+$ would also have a mass/charge ratio of 28.

In the last diagram, ion stream A is most deflected - it will contain ions with the smallest mass/charge ratio. Ion stream C is the least deflected - it contains ions with the greatest mass/charge ratio.

It makes it simpler to talk about this if we assume that the charge on all the ions is $1+$. Most of the ions passing through the mass spectrometer will have a charge of $1+$, so that the mass/charge ratio will be the same as the mass of the ion. Assuming $1+$ ions, stream A has the lightest ions, stream B the next lightest and stream C the heaviest. Lighter ions are going to be more deflected than heavy ones.

Detection

Only ion stream B makes it right through the machine to the ion detector. The other ions collide with the walls where they will pick up electrons and be neutralised. Eventually, they get removed from the mass spectrometer by the vacuum pump.



When an ion hits the metal box, its charge is neutralized by an electron jumping from the metal on to the ion (right hand diagram). That leaves a space amongst the electrons in the metal, and the electrons in the wire shuffle along to fill it. A flow of electrons in the wire is detected as an electric current which can be amplified and recorded. The more ions arriving, the greater the current.

Detecting the other ions

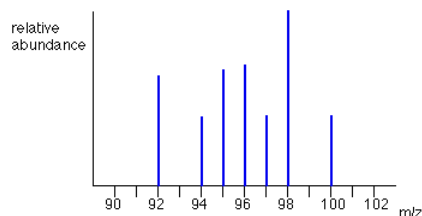
How might the other ions be detected - those in streams A and C which have been lost in the machine?

Remember that stream A was most deflected - it has the smallest value of m/z (the lightest ions if the charge is $1+$). To bring them on to the detector, you would need to deflect them less - by using a smaller magnetic field (a smaller sideways force). To bring those with a larger m/z value (the heavier ions if the charge is $+1$) on to the detector you would have to deflect them more by using a larger magnetic field.

If you vary the magnetic field, you can bring each ion stream in turn on to the detector to produce a current which is proportional to the number of ions arriving. The mass of each ion being detected is related to the size of the magnetic field used to bring it on to the detector. The machine can be calibrated to record current (which is a measure of the number of ions) against m/z directly. The mass is measured on the ^{12}C scale.

What the mass spectrometer output looks like

The output from the chart recorder is usually simplified into a "stick diagram". This shows the relative current produced by ions of varying mass/charge ratio. The stick diagram for molybdenum looks like this:



You may find diagrams in which the vertical axis is labeled as either "relative abundance" or "relative intensity". Whichever is used, it means the same thing. The vertical scale is related to the current received by the chart recorder - and so to the number of ions arriving at the detector: the greater the current, the more abundant the ion.

As you will see from the diagram, the commonest ion has a mass/charge ratio of 98. Other ions have mass/charge ratios of 92, 94, 95, 96, 97 and 100. That means that molybdenum consists of 7 different isotopes. Assuming that the ions all have a charge of $1+$, that means that the masses of the 7 isotopes on the carbon-12 scale are 92, 94, 95, 96, 97, 98 and 100.

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CHAPTER OVERVIEW

2.3.1: High-Performance Liquid Chromatography

High performance liquid chromatography has proven itself to very useful in many scientific fields, yet forces scientists to consistently choose between speed and resolution. Ultra High Performance Liquid Chromatography (UHPLC) eliminates the need to choose and creates a highly efficient method that is primarily based on small particle separations.

Topic hierarchy

- 2.3.1.1: Scope of HPLC
- 2.3.1.2: Column Efficiency in LC
- 2.3.1.3: Instrumentation
- 2.3.1.4: Partition Chromatography
- 2.3.1.5: Absorption Chromatography
- 2.3.1.6: Ion Chromatography
- 2.3.1.7: Size-Exclusion Chromatography
- 2.3.1.8: Affinity Chromatography
- 2.3.1.9: Thin-Layer Chromatography

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2.3.1.6: Ion Chromatography

In ion-exchange chromatography (IEC) the stationary phase is a cross-linked polymer resin, usually divinylbenzene cross-linked polystyrene, with covalently attached ionic functional groups. As shown in Figure 1 for a styrene–divinylbenzene co-polymer modified for use as an ion-exchange resin, the ion-exchange sites—indicated here by R and shown in blue—are mostly in the *para* position and are not necessarily bound to all styrene units. The cross-linking is shown in red. The counterions to these fixed charges are mobile and can be displaced by ions that compete more favorably for the exchange sites.

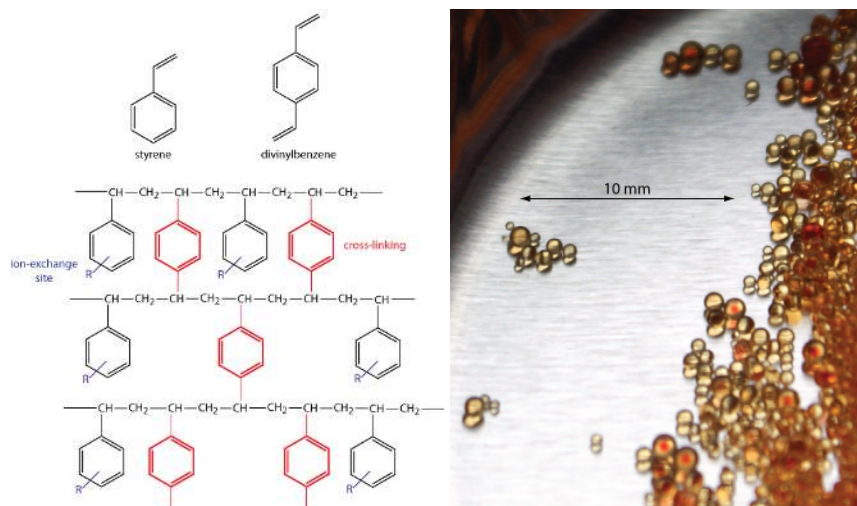


Figure 1: (left) styrene–divinylbenzene co-polymer modified for use as an ion-exchange resin. (right) The photo here shows an example of ion-exchange polymer beads. These beads are approximately 0.30–0.85 mm in diameter. Resins for use in ion-exchange chromatography are smaller, typically 5–11 μm in diameter.

Imagine if we had a tube whose surfaces were coated with an immobilized cation. These would have electrostatic attraction for anions. If a solution containing a mixture of positively and negatively charged groups flows through this tube, the anions would preferentially bind, and the cations in the solution would flow through (Figure 2).

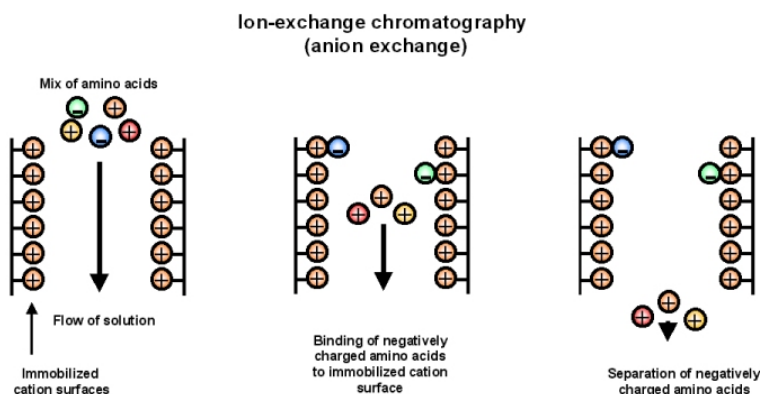
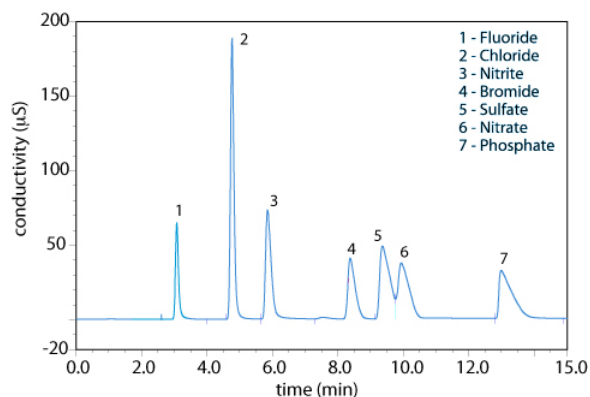


Figure 2: Separating action of amino acids in an anion exchange column.

- This is the basis of ion exchange chromatography. The example above is termed "anion exchange" because the inert surface is interacting with anions
- If the immobile surface was coated with anions, then the chromatography would be termed "cation exchange" chromatography (and cations would selectively bind and be removed from the solution flowing through)
- Strength of binding can be affected by pH, and salt concentration of the buffer. The ionic species "stuck" to the column can be removed (i.e. "eluted") and collected by changing one of these conditions. For example, we could lower the pH of the buffer and protonate anions. This would eliminate their electrostatic attraction to the immobilized cation surface. Or, we could increase the salt concentration of the buffer, the anions in the salt would "compete off" bound anions on the cation surface.

Ion-exchange chromatography is an important technique for the analysis of anions and cations in environmental samples. For example, an IEC analysis for the anions F^- , Cl^- , Br^- , NO_2^- , NO_3^- , PO_4^{3-} , and SO_4^{2-} takes approximately 15 minutes. A complete analysis of the same set of anions by a combination of potentiometry and spectrophotometry requires 1–2 days.



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2.3.1.7: Size-Exclusion Chromatography

Steric exclusion chromatography is a technique that separates compounds solely on the basis of size. In order for the results of steric exclusion separations to be meaningful, there can be no directed forces between the compounds being separated and the surface of the particles used as the stationary phase. Instead, the particles are prepared with well-characterized pore sizes. Figure 50 shows a particle with one pore in it. Figure 50 also shows representations for several molecules of different size (note, the pore size and molecule size are not representative of the scale that would exist in real steric exclusion phases – the pore is bigger than would actually occur and the molecules would never have sizes on the order of that of the particle).

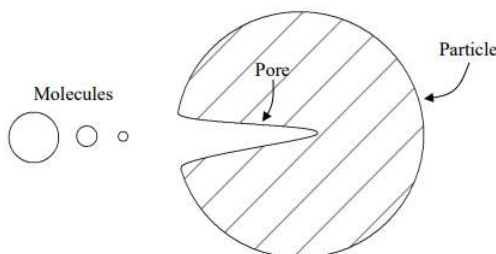
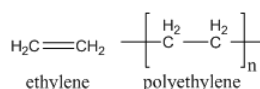


Figure 50. Representation of a particle with a pore.

The smallest molecule is small enough to fit entirely into all regions of the pore. The largest molecule is too big to fit into the pore. The intermediate sized one can only sample some of the pore volume. Provided these molecules have no interaction with the surface of the particle and are only separated on the basis of how much of the pore volume they can sample, the largest molecule would elute first from the column and the smallest one last.

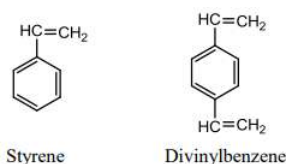
Suppose we took a molecule that was even larger than the biggest one in the picture above. Note that it would not fit into any of the pores either, and would elute with the biggest in the picture. If we wanted to separate these large species, we would need a particle with even larger pores. Similarly, if we took a smaller molecule than the smallest pictured above, it would sample all of the pore volume and elute with the smallest one pictured above. To separate these two compounds, we would need particles with smaller pores.

Steric exclusion chromatography requires large compounds, and is not generally effective on things with molecular weights of less than 1000. It is commonly used in biochemistry for the separation of proteins and nucleic acids. It is also used for separation or characterization purposes in polymer chemistry (a polymer is a large compound prepared from repeating monomeric units – polyethylene is a long, linear polymer made of repeating ethylene units).



One critical question is how to remove the possibility of an attractive force between the compounds being separated and the surface of the porous particles. The way this is done is to use a particle with very different properties than the compound being separated, and to use a solvent that the solutes are very soluble in. For example, if we want to separate proteins or nucleic acids, which are polar and very soluble in water, we would need to use porous particles made from an organic material that had a relatively non-polar surface. If we wanted to separate polyethylenes of different chain lengths, which are non-polar, we would dissolve the polyethylene in a non-polar organic solvent and use porous particles made from a material that had a highly polar surface. This might involve the use of a polydextran (carbohydrate), which has hydroxyl groups on the surface of the pores.

The classic organic polymer that has been used to prepare porous particles for the steric exclusion separation of water-soluble polymers is a co-polymer of styrene and divinylbenzene.



If we just had styrene, and conducted a polymerization, we would get a long, single-bonded chain of carbon atoms with phenyl rings attached to it. Linear polystyrenes are soluble in certain non-polar organic solvents. The divinylbenzene acts as a cross-linking agent that bridges individual styrene chains (Figure 51). A typical ratio of styrene to divinylbenzene is 12:1. The cross-linking serves to make the polymer into particles rather than linear chains. The cross-linked polymer is an insoluble material with a very high molecular weight. By controlling the reaction conditions including the amount of cross-linker and rate of reaction, it is possible to make polystyrenes with a range of pore sizes.

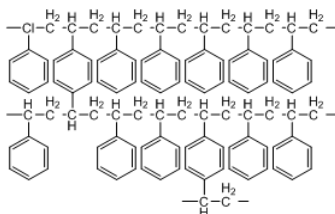


Figure 51. Cross-linked styrene-divinylbenzene copolymer.

If we think about what is inside the column, there are four important volume terms to consider. One is the total volume of the column (V_T). The second is the interstitial volume between the particles (V_I). The third is the volume of all of the pores (V_P). The last one is the actual volume occupied by the mass of the material that makes up the particles.

It turns out that V_I is usually about 0.3 of V_T for a column. There is simply no way to crunch the particles closer together to reduce this term appreciably.

$$V_I = 0.3V_T \quad (2.3.1.7.1)$$

It turns out that the maximum pore volume that can be achieved is about 0.4 of V_T . If you make this larger, the particles have more pores than structure, become excessively fragile, and get crushed as you try to push a liquid mobile phase through the column.

$$V_P = 0.4V_T \quad (2.3.1.7.2)$$

Totaling this up, we can write that:

$$V_I + V_P = 0.7V_T \quad (2.3.1.7.3)$$

Since there are no directed forces in a steric exclusion column, this means that all of the separation must happen in one column volume, or in $0.7 V_T$. If we then showed a chromatogram for a series of compounds being separated on a steric exclusion, it might look like that shown in Figure 52.

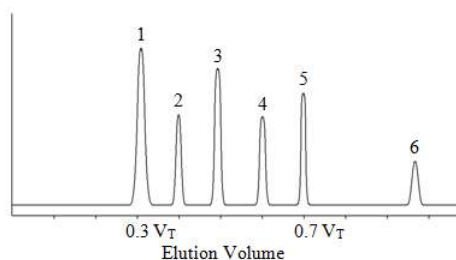


Figure 52. Representation of a chromatogram on a steric exclusion column.

The peak labeled 1, which has the shortest retention time ($0.3 V_T$), corresponds to all molecules in the sample that had a size that was too large to fit into the pores. It cannot be known whether this is one compound or a mixture of large compounds. The peak labeled 5, which has the longest retention time ($0.7 V_T$), corresponds to all molecules in the sample that had a size small enough to sample all of the pore volume. It cannot be known whether it is one compound or a mixture of two or more small compounds. The peaks labeled 2 through 4 sample some of the pore volume depending on their size. The peak labeled 6 elutes with a retention volume that is greater than $0.7 V_T$. How would we explain the elution volume of this peak? The only way we can do so is if there are attractive forces between this molecule and the surface of the particles. This is a serious problem and we would need to examine this system and eliminate the cause of this attractive force.

Note that this method separates things on the basis of their size. What people try to do when using steric exclusion chromatography is equate size with molecular weight. The two representations for the shape of a molecule shown in Figure 53 point out a potential problem with equating size with molecular weight. One molecule is spherical. The other is rod shaped. The spherical molecule probably has a larger molecular weight, because it does occupy more volume. However, the size of a molecule is determined by what is known as its hydrodynamic radius. This is the volume that the molecule sweeps out in space as it tumbles. If you took the rod shaped molecule below and allowed it to tumble, you would see that its size is essentially comparable to that of the spherical molecule.

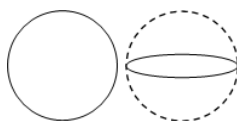


Figure 53. Representation of the molecular size of spherical (left) and rod-shaped (right) molecules.

When performing steric exclusion chromatography, you need to use a series of molecular weight standards. It is essential that these standards approximate the molecular features and shapes of the molecules you are analyzing. If you are analyzing proteins, you need to use proteins as standards. The same would apply to nucleic acids, or to particular organic polymers. Figure 54 shows the typical outcome of the calibration of elution time with molecular weight (always plotted on a log scale) for a steric exclusion column.

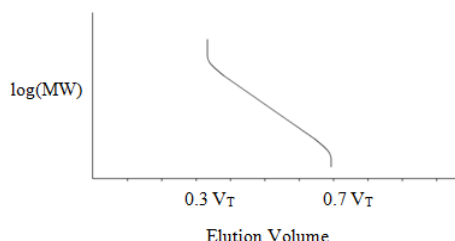


Figure 54. Generalized calibration curve for a steric exclusion column.

Note that excessively large molecules never enter the pores and elute together at $0.3 V_T$. Excessively small molecules sample all of the pore volume and elute at $0.7 V_T$. There is then some range of size (or molecular weight) where the compounds sample some of the pore volume and elute at volumes between 0.3 and $0.7 V_T$.

The plots that would occur for particles with the next larger (dashed line) and smaller pore sizes (dotted line) are shown in Figure 55.

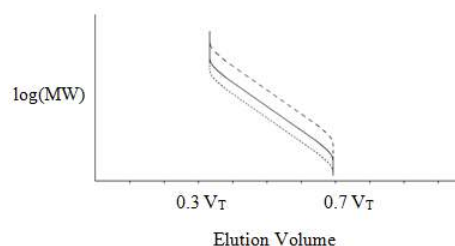


Figure 55. Calibration curves for a steric exclusion column. The dashed line is the column with the largest pore sizes. The dotted line is the column with the smallest pore sizes.

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2.3.1.8: Affinity Chromatography

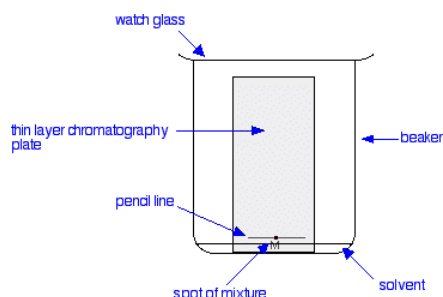
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2.3.1.9: Thin-Layer Chromatography

Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle. They all have a stationary phase (a solid, or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates.

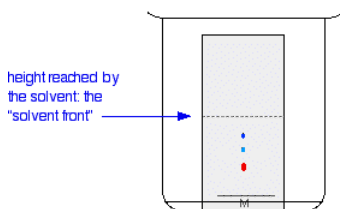
Thin layer chromatography is done exactly as it says - using a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The silica gel (or the alumina) is the stationary phase. The stationary phase for thin layer chromatography also often contains a substance which fluoresces in UV light - for reasons you will see later. The mobile phase is a suitable liquid solvent or mixture of solvents.

We'll start with a very simple case - just trying to show that a particular dye is in fact a mixture of simpler dyes.



A pencil line is drawn near the bottom of the plate and a small drop of a solution of the dye mixture is placed on it. Any labelling on the plate to show the original position of the drop must also be in pencil. If any of this was done in ink, dyes from the ink would also move as the chromatogram developed. When the spot of mixture is dry, the plate is stood in a shallow layer of solvent in a covered beaker. It is important that the solvent level is below the line with the spot on it.

The reason for covering the beaker is to make sure that the atmosphere in the beaker is saturated with solvent vapor. To help this, the beaker is often lined with some filter paper soaked in solvent. Saturating the atmosphere in the beaker with vapor stops the solvent from evaporating as it rises up the plate. As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates and the mixture is separated into different coloured spots.

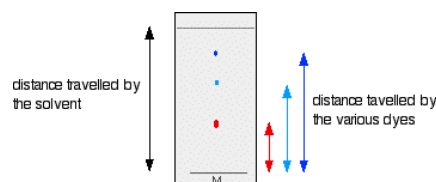


The diagram shows the plate after the solvent has moved about half way up it. The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the dye components for this particular combination of solvent and stationary phase.

Measuring R_f values

If all you wanted to know is how many different dyes made up the mixture, you could just stop there. However, measurements are often taken from the plate in order to help identify the compounds present. These measurements are the distance traveled by the solvent, and the distance traveled by individual spots. When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

These measurements are then taken:



The R_f value for each dye is then worked out using the formula:

$$R_f = \frac{\text{distance traveled by sample}}{\text{distance traveled by solvent}}$$

For example, if the red component traveled 1.7 cm from the base line while the solvent had traveled 5.0 cm, then the R_f value for the red dye is:

$$\begin{aligned} R_f &= \frac{1.7}{5.0} \\ &= 0.34 \end{aligned}$$

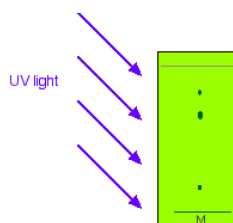
If you could repeat this experiment under exactly the same conditions, then the R_f values for each dye would always be the same. For example, the R_f value for the red dye would always be 0.34. However, if anything changes (the temperature, the exact composition of the solvent, and so on), that is no longer true. You have to bear this in mind if you want to use this technique to identify a particular dye. We'll look at how you can use thin layer chromatography for analysis further down the page.

What if the substances you are interested in are colorless?

There are two simple ways of getting around this problem.

Using fluorescence

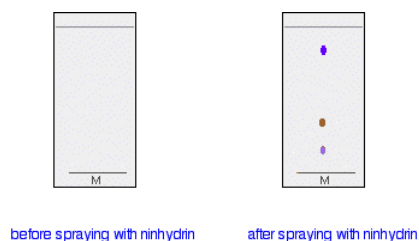
You may remember that I mentioned that the stationary phase on a thin layer plate often has a substance added to it which will fluoresce when exposed to UV light. That means that if you shine UV light on it, it will glow. That glow is masked at the position where the spots are on the final chromatogram - even if those spots are invisible to the eye. That means that if you shine UV light on the plate, it will all glow apart from where the spots are. The spots show up as darker patches.



While the UV is still shining on the plate, you obviously have to mark the positions of the spots by drawing a pencil circle around them. As soon as you switch off the UV source, the spots will disappear again.

Showing the spots up chemically

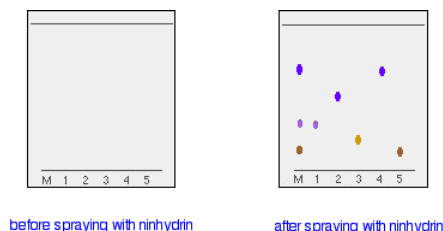
In some cases, it may be possible to make the spots visible by reacting them with something which produces a coloured product. A good example of this is in chromatograms produced from amino acid mixtures. The chromatogram is allowed to dry and is then sprayed with a solution of ninhydrin. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple.



In another method, the chromatogram is again allowed to dry and then placed in an enclosed container (such as another beaker covered with a watch glass) along with a few iodine crystals. The iodine vapor in the container may either react with the spots on the chromatogram, or simply stick more to the spots than to the rest of the plate. Either way, the substances you are interested in may show up as brownish spots.

Using thin layer chromatography to identify compounds

Suppose you had a mixture of amino acids and wanted to find out which particular amino acids the mixture contained. For simplicity we'll assume that you know the mixture can only possibly contain five of the common amino acids. A small drop of the mixture is placed on the base line of the thin layer plate, and similar small spots of the known amino acids are placed alongside it. The plate is then stood in a suitable solvent and left to develop as before. In the diagram, the mixture is M, and the known amino acids are labelled 1 to 5.

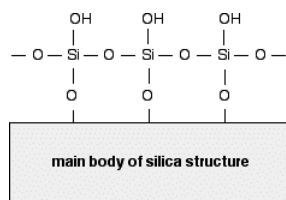


The left-hand diagram shows the plate after the solvent front has almost reached the top. The spots are still invisible. The second diagram shows what it might look like after spraying with ninhydrin. There is no need to measure the R_f values because you can easily compare the spots in the mixture with those of the known amino acids - both from their positions and their colours. In this example, the mixture contains the amino acids labelled as 1, 4 and 5. And what if the mixture contained amino acids other than the ones we have used for comparison? There would be spots in the mixture which didn't match those from the known amino acids. You would have to re-run the experiment using other amino acids for comparison.

How does thin layer chromatography work?

The stationary phase - silica gel

Silica gel is a form of silicon dioxide (silica). The silicon atoms are joined via oxygen atoms in a giant covalent structure. However, at the surface of the silica gel, the silicon atoms are attached to -OH groups. So, at the surface of the silica gel you have Si-O-H bonds instead of Si-O-Si bonds. The diagram shows a small part of the silica surface.



The surface of the silica gel is very polar and, because of the -OH groups, can form hydrogen bonds with suitable compounds around it as well as van der Waals dispersion forces and dipole-dipole attractions.

The other commonly used stationary phase is alumina - aluminium oxide. The aluminium atoms on the surface of this also have -OH groups attached. Anything we say about silica gel therefore applies equally to alumina.

What separates the compounds as a chromatogram develops?

As the solvent begins to soak up the plate, it first dissolves the compounds in the spot that you have put on the base line. The compounds present will then tend to get carried up the chromatography plate as the solvent continues to move upwards. How fast the compounds get carried up the plate depends on two things:

- How soluble the compound is in the solvent. This will depend on how much attraction there is between the molecules of the compound and those of the solvent.

- How much the compound sticks to the stationary phase - the silica gel, for example. This will depend on how much attraction there is between the molecules of the compound and the silica gel.

Suppose the original spot contained two compounds - one of which can form hydrogen bonds, and one of which can only take part in weaker van der Waals interactions. The one which can hydrogen bond will stick to the surface of the silica gel more firmly than the other one. We say that one is adsorbed more strongly than the other. Adsorption is the name given to one substance forming some sort of bonds to the surface of another one.

Adsorption isn't permanent - there is a constant movement of a molecule between being adsorbed onto the silica gel surface and going back into solution in the solvent. Obviously the compound can only travel up the plate during the time that it is dissolved in the solvent. While it is adsorbed on the silica gel, it is temporarily stopped - the solvent is moving on without it. That means that the more strongly a compound is adsorbed, the less distance it can travel up the plate.

In the example we started with, the compound which can hydrogen bond will adsorb more strongly than the one dependent on van der Waals interactions, and so won't travel so far up the plate.

What if both components of the mixture can hydrogen bond?

It is very unlikely that both will hydrogen bond to exactly the same extent, and be soluble in the solvent to exactly the same extent. It isn't just the attraction of the compound for the silica gel which matters. Attractions between the compound and the solvent are also important - they will affect how easily the compound is pulled back into solution away from the surface of the silica. However, it may be that the compounds don't separate out very well when you make the chromatogram. In that case, changing the solvent may well help - including perhaps changing the pH of the solvent. This is to some extent just a matter of trial and error - if one solvent or solvent mixture doesn't work very well, you try another one. (Or, more likely, given the level you are probably working at, someone else has already done all the hard work for you, and you just use the solvent mixture you are given and everything will work perfectly!)

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CHAPTER OVERVIEW

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CHAPTER OVERVIEW

3.1: An Introduction to Electroanalytical Chemistry

Electrochemistry can be defined as the *study of phenomena at electrified interfaces*. As such, two things are fundamental to electrochemical measurements:

1. Interface: boundary between two distinct, chemically different phases
2. Electric field: existing across the interface

Generally, the interface occurs between a metallic conductor (the electrode) and a fluid, ionic conductor (the solution). The electric field usually results as a consequence of contact between a solid electrode and the solution. In electrochemistry, the electric field is most often one that is under user control, for example by utilizing a device called a *potentiostat*, which will be discussed in a later section.

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3.1.1: Electrochemical Cells

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3.1.2: Potentials in Electroanalytical Cells

Potential can be described as the work required to move an electron or other point reference charge from an infinite distance away to a point of interest – inside of a metallic electrode, for example. The magnitude of the potential at the surface of an electrode depends on the *excess* charge that exists there above that of the metal alone. An external power supply is capable of forcing excess electrons into or out of the electrode, leading to a non-equilibrium condition at the interface. If a large excess of electrons is present in the electrode, the potential of the electrode is caused to be very *negative*, with the energy of the electrons in the electrode being very *high*. Likewise, excess positive charge caused by the removal of electrons from the electrode leads to a *positive* potential at the electrode, and low electron energy.

The measurement of electrode energy requires that a reference point be established, and that individual electrode energies be taken relative to a potential that is fixed. This is normally done by introducing a second electrode, called the *reference electrode*, whose chemical composition is fixed and its potential energy unchanging. Thus, the term *potential*, in an electrochemical sense means the *voltage difference* between two electrodes, and is denoted “E”, with units of volts. The arrangement of a metal electrode immersed in a solution of charged electrolyte along with a reference electrode is referred to as an *electrochemical cell* (**Figure 1**), and E the *cell potential*.

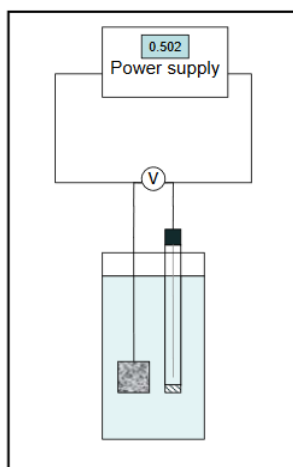


Figure 1

Charge movement can occur between the metal electrode and a solution species when the cell potential is sufficient to either promote an electron into the lowest unoccupied molecular orbital (LUMO) of the solution species (called a *reduction*) or to allow movement from the highest occupied molecular orbital of the solution species (HOMO) into the metal electrode (called an oxidation). In either case, the electrons involved in charge movement end up in a place offering them the lowest available energy (“electrons are lazy”). This process is illustrated in **Figure 2**.

Because work is being done by the metal electrode, it is commonly referred to as the *working electrode*. In the simplest description, the potential at which an electron transfer takes place can be related to the overall free energy change (ΔG) of the reaction by

$$\Delta G = -n F E \quad (3.1.2.1)$$

where n is the stoichiometric *number of electrons* involved in the reaction and F is the *Faraday constant* which relates the total charge (in coulombs, C) of the reaction to the amount of product formed (96,485 C/mole for $n = 1$).

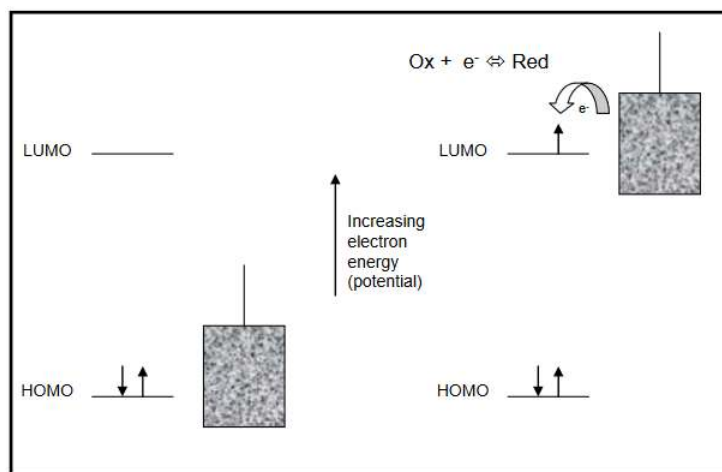
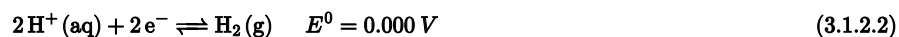


Figure 2

There exists a well-defined, critical energy required for any electrode process, called the E^0 . Tables of E^0 values are available for many redox couples. The potentials found there were measured for each half-cell with all species present at unit activity relative to the standard hydrogen electrode. The standard hydrogen electrode (SHE) is a half-cell composed of an inert solid electrode like platinum on which hydrogen gas is adsorbed at unit activity, immersed in a solution containing hydrogen ions at unit activity. The half-cell reaction is given by



and it has arbitrarily been assigned a half-cell potential of 0.000 V.

At this point, it is adequate to view the oxidation/reduction process of a solution species as a function of potential applied to the working electrode. If the working electrode is more *positive* than the E^0 for the solution species, the oxidized form of the species is stable at the electrode surface. Further, if the working electrode is more *negative* than the E^0 the reduced form of the species will be stable. Together, the oxidized and reduced forms of the species represent a *redox couple*.

Electron transfers of the type just described in which electrons are transferred across the metal-solution interface with a resulting oxidation or reduction of a solution species are called *faradaic processes*. Study of these charge transfer reactions is the goal of most techniques in analytical electrochemistry. Unfortunately, the complexity of the interfacial system is such that even in the absence of faradaic electron transfer, other processes do occur that can affect electrode behavior. These processes include adsorption, desorption, and charging of the interface as a result of changing electrode potential. These are called *nonfaradaic processes*, and lead us to a short discussion of what is termed the *electrical double layer*.

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3.1.3: Electrode Potentials

Consider a solution containing only inert electrolyte (no faradaic redox couple), in which no charge passes across the electrode-solution interface over a given range of potential. Under these conditions, a change in potential at the working electrode from its equilibrium value necessarily causes a charge imbalance across the interface which must be neutralized by rearrangement of charged species in the solution near the electrode surface. In this sense, the interfacial region can be represented by the two plates of a capacitor, whose *capacitance* is described by

$$\frac{q}{E} = C \quad (3.1.3.1)$$

where q is the charge in coulombs stored on the capacitor, E is the potential across the capacitor in volts, and C is the capacitance in Farads (F). The situation for one value of applied potential in which the electrode surface is negatively charged is illustrated in **Figure 3**.

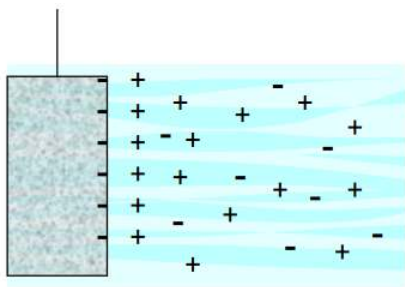


Figure 3

Each time the potential at the electrode is changed, charge will flow (current) at the interface until the capacitance equation above has been satisfied, resulting in what is referred to as a *charging current*. In actual fact, of course, the situation between the charged electrode and charged species in solution is significantly more complex, with the capacitor model being only a satisfying approximation.

The structure of the so-called double layer actually contains several layers on the solution side, whose thickness and composition greatly affect electron transfer to faradaic solution species. The inner-most or *compact* layer is composed of solvent, ions, and molecules that are not fully solvated, and which are said to be *specifically adsorbed* to the electrode surface. The center of this adsorbed layer defines the *inner Helmholtz plane*, which represents the closest approach of solvated ions to the surface of the electrode. These solvated ions interact with the surface of the electrode in a long-range electrostatic fashion only, and are said to be *nonspecifically adsorbed*. The center of this second layer defines the *outer Helmholtz plane*, beyond which these ions are distributed in the *diffuse layer*, extending into the bulk of the solution only a few hundred Angstroms.² Beyond the diffuse layer, ions are not subject to electrostatic effects initiated by the charged electrode surface. Interested readers will find an extensive discussion of this topic in any good electrochemistry textbook, like that written by Bard and Faulkner.²

Because potential measurement requires two separate electrodes, two distinct interfacial regions exist during the measurement. The movement of charge across these interfacial regions in the presence of a redox couple is the basis of most commonly employed electrochemical methods.

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CHAPTER OVERVIEW

3.2: Potentiometry

If you have ever used a pH meter, then you have already performed potentiometry, an electrochemical method in which the potential of an electrochemical cell is measured while little to no current is passed through the sample. In a potentiometric measurement, an indicator electrode responds to changes in the activity, or “effective concentration” of the analyte. A potential, or voltage, that develops at the interface between the electrode and the analyte solution is measured relative to a reference electrode. This potential will be proportional to the amount of analyte in the sample. https://chem.libretexts.org/Core/Ana..._Potentiometry

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- [3.2.2: Reference Electrodes](#)
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3.2.1: General Principles

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3.2.2: Reference Electrodes

It should be clear by now that at least two electrodes are necessary to make a potential measurement. As Kissinger and Bott have so perfectly expressed, “electrochemistry with a single electrode is like the sound of one hand clapping” (<http://currentseparations.com/issues/20-2/20-2d.pdf>). In potentiometry, those two electrodes are generally called the **indicator** electrode and the **reference** electrode. The indicator electrode possesses some characteristic that allows it to selectively respond to changes in the activity of the analyte being measured. For the measured potential to have meaning in this context, the reference electrode must be constructed so that its composition is fixed and its response is stable over time, with observed changes in measured potential due solely to changes in analyte concentration.

You are probably familiar with tables of standard reduction potentials from a general chemistry course. The standard reduction potential, or E^0 , allows you to predict the ease with which a half-cell reaction occurs relative to other half-reactions. (For a review of standard potentials and electrochemical cells, you can access the companion “[Concepts](#)” module). Values of E^0 are most often reported as the potential measured in an electrochemical cell for which the **standard hydrogen electrode** is used as a reference.

The *standard hydrogen electrode*, or SHE, is composed of an inert solid like platinum on which hydrogen gas is adsorbed, immersed in a solution containing hydrogen ions at unit activity. The half-cell reaction for the SHE is given by



and the half-cell potential arbitrarily assigned a value of zero ($E^0 = 0.000 \text{ V}$).

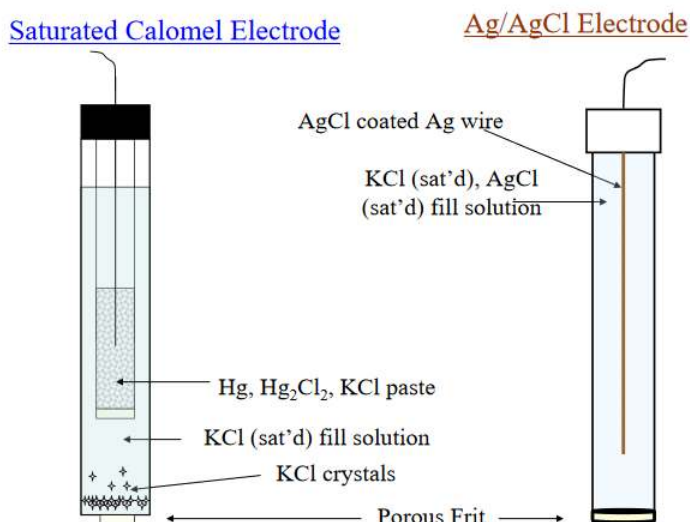
Practical application of the SHE is limited by the difficulty in preparing and maintaining the electrode, primarily due to the requirement for $\text{H}_2(\text{g})$ in the half-cell. Most potentiometric methods employ one of two other common reference half-cells – the **saturated calomel electrode** (SCE) or the **silver-silver chloride electrode** (Ag/AgCl).

1. Saturated Calomel Electrode (SCE)

The SCE is a half cell composed of *mercurous chloride* (Hg_2Cl_2 , *calomel*) in contact with a mercury pool. These components are either layered under a saturated solution of potassium chloride (KCl) or within a fritted compartment surrounded by the saturated KCl solution (called a double-junction arrangement). A platinum wire is generally used to allow contact to the external circuit. The half reaction is described by



with an E^0 value of +0.244 V. A common arrangement for the SCE is shown below, left side. In this arrangement, a paste of the calomel and solution that is saturated with KCl.



The solution over the paste is also saturated with KCl, with some solid KCl crystals present. Contact to the measurement cell is made through a porous glass frit or fiber which allows the movement of ions, but not the bulk solution. In many electrodes designed for potentiometry, the reference half cell is contained within the body of the sensing electrode. This arrangement is referred to as a “combination” electrode.

2. Silver/Silver Chloride (Ag/AgCl)

The silver/silver chloride reference electrode is composed of a silver wire, sometimes coated with a layer of solid silver chloride, immersed in a solution that is saturated with potassium chloride and silver chloride. The pertinent half reaction is



with a value for E^0 of +0.222 V. The actual potential of the half-cell prepared in this way is +0.197 V vs SHE, which arises because in addition to KCl, AgCl also contributes to the chloride activity, which is not exactly unity. A schematic of the Ag/AgCl reference electrode is shown at right in the previous figure.

Both the SCE and the Ag/AgCl reference electrodes offer stable half-cell potentials that do not change over time or with temperature. In addition, the loss of electrolyte to evaporation does not change the saturated nature of the solution, nor the potential. One must be aware that the contact junctions of the half cells by nature slowly leak fill solution into the external solution in which they are found. As such, there are instances where measurements of certain ions, like chloride, might be affected by the ions introduced to the measurement solution by leakage. The doublejunction design prevents this problem by placing a second solution between the reference half cell and the measurement solution. This of course adds a second *junction potential*, a topic covered elsewhere in this module.

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