CHAPTER 4. SPECTROSCOPY

4.1 THE INTERACTION OF LIGHT AND MATTER

When a chemist uses the term "spectroscopy", it is usually taken to mean the study of the interaction of light and matter. The use of light (strictly speaking electromagnetic radiation) in its many forms to probe matter gives the chemist one of the most powerful techniques available for determining the characteristics of the material under examination. Thus a fairly thorough introduction to some of the spectroscopic techniques most commonly used in analytical chemistry will be presented. Before beginning this topic some time must be taken to discuss the nature of light. The difficulty here is that there is no single, simple description of light that satisfies all situations. There are two descriptions that are usually given, and the one that is used in any particular case is the one that describes most readily the interactions which occur in that case.

4.1.1 THE WAVE DESCRIPTION OF LIGHT

As noted above light is more accurately referred to as electromagnetic radiation, indicating that it has both electric and magnetic properties. These properties in turn behave in a wave fashion. If one placed an electric charge and a tiny monopolar magnet (if such a thing existed) in the path of a light ray, one would observe that the charge would oscillate back and forth in one plane and the magnet would oscillate back and forth in a plane perpendicular to that of the charge as the ray passed (see Figure 4-1). In addition the direction of the light ray would be perpendicular to the motions of both the charge and the magnet. This motion would be the same as that of a piece of wood on water disturbed by water waves. Thus one would conclude that light could be described as an oscillating electric field perpendicular to an oscillating magnetic field, and these fields would be perpendicular to the direction of the wave propagation.

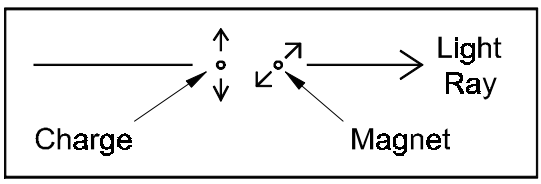

Figure 4-1

If the wave could be "photographed" at a single instant in time, the result would be as shown in Figure 4-2. Both the electric and magnetic fields are sinusoidal in shape as a function of distance. The distance between two similar places on the wave, *e.g.* two peaks or two zero points, is called the wavelength. The usual dimensions of wavelength are metres or centimetres.

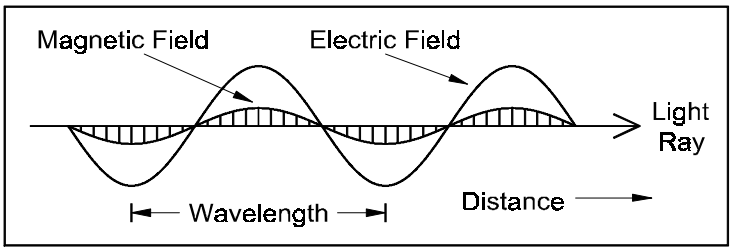

Figure 4-2

If one were to observe the light wave from one point in space as a function of time, a very similar picture could be drawn, but now the X axis (abscissa) would have units of time rather than distance, as shown in Figure 4-3. Here the time between two similar points on the wave is called one period or cycle of the waveform (units of seconds). The

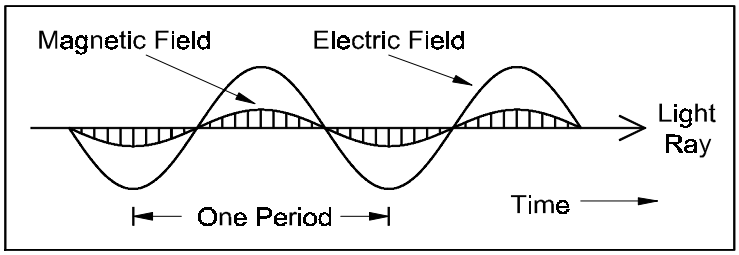

Figure 4-3

number of cycles that pass an observer per second as the wave moves along is called the frequency with units of sec^{-1}. In honour of a scientist that influenced the development of electromagnetic theory, the units of frequency are often called Hertz (one Hertz = one sec^{-1}).

A simple relationship exists for all wave motion (light, sound, *etc*.) between the velocity, V, the frequency, ν, and the wavelength, λ.

$$V = \nu\lambda \qquad (4\text{-}1)$$

For a light wave the symbol for velocity is usually taken as "*c*". Thus

$$c = \nu\lambda = 2.998\text{x}10^{8} \text{ m/s (in vacuum)} \qquad (4\text{-}2)$$

For all electromagnetic radiation ν *is constant*, however, c and λ may change when the light wave passes from one medium to another, *e.g.* from air into water.

4.1.2 THE PARTICLE DESCRIPTION OF LIGHT

Under certain conditions light behaves as though it were a stream of particles called photons. These are rather strange particles because they cannot exist unless they are in motion (their velocity is that of the light ray). The energy of a particle, E, is related to the frequency of the light ray according to

$$E = h\nu = hc/\lambda \qquad (4\text{-}3)$$

where $h = 6.626\text{x}10^{-34}$ Js (Planck's constant)

Note that the particle description still involves wave properties, *e.g.* the frequency. As frequency increases (wavelength decreases, since velocity is usually fairly constant), energy increases. Thus one could use either the energy, the frequency, or the wavelength to describe different light waves. Since either the energy or the frequency are invariant for a given wave, it would be best to use one of these. However, for the "type" of light to be discussed in this text, the wavelength is most commonly used to describe or classify the light being employed in an experiment.

In theory light can have any frequency (or energy) from zero to nearly infinity. This gives rise to a wide variety of electromagnetic waves. For example, radio waves (10^{6} Hz) and X-rays (10^{18} Hz) are both "light" waves. All the different kinds of light waves are said to make up the electromagnetic spectrum. Photons in different regions of the spectrum interact with matter in different ways.

Only a very small part of the electromagnetic spectrum will be discussed, the visible and ultraviolet regions. For obvious reasons these were the first regions to be used experimentally and are still very important in chemical analysis. Table 4-1 gives the various characteristics of light in this region of the spectrum.

Table 4-1

Characteristic	Values						Units
Colour	(ultraviolet)	blue	green	yellow	orange	red	
Wavelength	200-400	400	450	500	600	700	nm
Frequency	$1.5\text{x}10^{15}$	$7.5\text{x}10^{14}$	$6.7\text{x}10^{14}$	$6\text{x}10^{14}$	$5\text{x}10^{14}$	$4.3\text{x}10^{14}$	Hz
Energy	$9.9\text{x}10^{-19}$	$5\text{x}10^{-19}$	$4.4\text{x}10^{-19}$	$4\text{x}10^{-19}$	$3.3\text{x}10^{-19}$	$2.8\text{x}10^{-19}$	J

The energy of one mole of "red" photons = ($6.0\text{x}10^{23}$ photons/mol)($2.8\text{x}10^{-19}$ J/photon) = 170 kJ/mol. That of one mole of ultraviolet (uv) photons (200 nm) is 590 kJ/mol. These energies are of

the same order of magnitude as those involved in chemical reactions. This might give a clue as to what happens when light in this region of the spectrum interacts with matter. Chemical reactions involve the rearrangement of valence electrons, and so will the interaction of visible and ultraviolet light with matter.

The colours of the different wavelengths of visible light are given in Table 4-1. It should be noted that white light is a mixture of all the different colours (or wavelengths) of light in the visible region of the spectrum and that the human eye is insensitive to ultraviolet light, which therefore has no colour.

4.1.3 ABSORPTION AND EMISSION OF VISIBLE AND UV LIGHT

It is most convenient to use the particle description of light when discussing the types of interactions between light and matter that are used in chemical analysis. In this text only two types of interactions, the absorption and the emission of light by matter, will be examined. The absorption of light will be discussed first. When light is absorbed, the photon completely disappears and its energy is converted to some form of energy in the material that absorbed the light. This additional energy in the absorbing material can take several forms:

(a) *kinetic* or *translational energy* (heat) - the atoms or molecules of the material would move more rapidly,

(b) *rotational energy* - the molecules would tumble more rapidly,

(c) *vibrational energy* - the atoms of the molecules would vibrate about their bonds more rapidly, and

(d) *electronic energy* - the valence electrons would be promoted to higher orbitals.

Atomic spectra

The interaction of light with atoms is less complex than with molecules, and this will be discussed first. The reason is simple, atoms do not possess rotational or vibrational energy, only translational and electronic energy. The energy of visible and uv photons is far higher than the translational energy observed at room temperature. Since there must be a match between the energy of the photon being absorbed and the type of energy being "created" in the absorber, visible and uv photons are not converted *directly* to translational energy in the types of interactions of interest to analytical chemists. Thus the energy of such photons must be converted to electronic energy in the atoms; that is, a valence electron must be promoted from a lower energy orbital to a higher energy orbital. The energies of such orbitals are quantized, which means that orbitals have fixed energies and that there are no energy levels with intermediate energies. The orbital energy level diagram for the simplest atom, hydrogen, is shown in Figure 4-4. In this figure "n" represents the principal quantum number and the arrows indicate the possible transitions that can occur when light is absorbed.

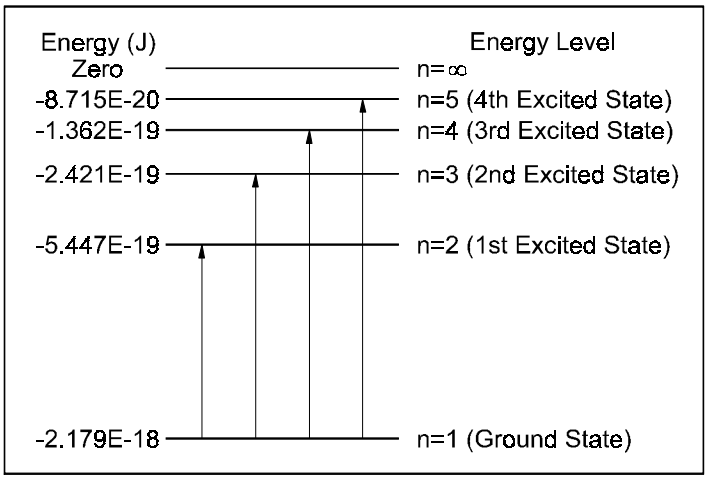

Figure 4-4

The lowest energy state, n = 1 for H, is called the ground state. In atoms this is the state with all electrons in their lowest energy orbitals. If an electron has been promoted to a higher orbital, *e.g.* n = 2 for H, this is called an excited state. These states are usually unstable and the electron will return to a lower energy orbital spontaneously (with the emission of light). When light is absorbed, the energy of the photon is used to promote a valence electron to a higher orbital, and the photon energy must match exactly the energy difference between the two orbitals.

$$E_{\text{photon}} = h\nu = \Delta E_{\text{orbitals}} = E_f - E_i \qquad (4\text{-}4)$$

where E_f and E_i are the final and initial orbital energies. If the light falling on the atoms does not have an energy exactly equal to the difference in orbital energies, it cannot cause an electron to change orbitals and *it cannot be absorbed*. In Figure 4-4 above the vertical arrows represent the energy differences between the orbitals of hydrogen. Thus light with photons of these energies will be absorbed, but no other light. The characteristics of the light absorbed by hydrogen atoms for the transitions in Figure 4-4 are listed in Table 4-2.

Table 4-2

Transition	n=1 → n=2	n=1 → n=3	n=1 → n=4	n=1 → n=5
Wavelength (nm)	121.6	102.6	97.3	95.0
Frequency (Hz)	2.74×10^{15}	2.92×10^{15}	3.08×10^{15}	3.17×10^{15}
Energy (kJ/mol)	984	1166	1229	1259

With these concepts in mind, what would happen if light of different wavelengths (different energies) were used to illuminate a sample of hydrogen atoms? One could plot the results of such an experiment as percent light absorbed *vs* wavelength. The results of such an experiment would look something like those shown in Figure 4-5. Such a plot is called an absorption spectrum (a plot of light absorbed *vs* wavelength). Note that only light at very specific wavelengths is absorbed. These bands of absorbed light are very narrow, so narrow that they are usually called absorption *lines*.

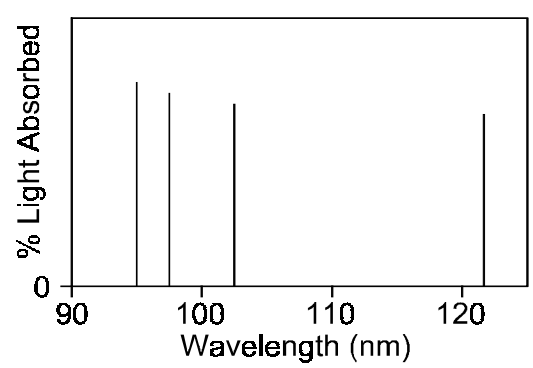

Figure 4-5

The lines shown in this diagram are called *resonance lines* because they originate in the ground state - that is the atom was in its ground state before absorbing the photon. At room temperature, and even at considerably higher temperatures, almost all atoms will normally be in their ground states. However, electronic transitions do not have to originate from the ground state. Thus the absorption spectrum for hydrogen is much more complex than shown in Figure 4-5. The spectra of other atoms are even more complex than that of hydrogen, because all other atoms have more than one valence electron, and these interact with one another. However, these spectra are still *line spectra* and are different for each element, thus allowing positive identification of each element to be made from such spectra.

Molecular spectra

The picture is much more complicated in the case of molecules. Molecules possess *rotational* and *vibrational* energy in addition to translational and electronic energy. Just as the electronic energy

levels are quantized, so are the vibrational and rotational energy levels. Thus there are *distinct* levels for each of these types of energy. In terms of energy scale the rotational energy levels are much closer together than the vibrational levels (it takes much less energy to make a molecule rotate faster than to make it vibrate more rapidly) and likewise the vibrational levels are much closer together than the electronic levels (it takes much less energy to cause more rapid vibration than to promote a valence electron to a higher state). The result of this is that these energies are reasonably independent of one another. In other words a molecule in the ground electronic state can vibrate and rotate very rapidly while one in an excited electronic state can vibrate and rotate slowly. Thus each electronic state has a whole set of vibrational states and each vibrational state has a whole set of rotational states. This gives rise to an energy level diagram similar to that shown in Figure 4-6. This stylized diagram is very approximate and is only meant to impart the general ideas involved with energy levels and the transitions between them.

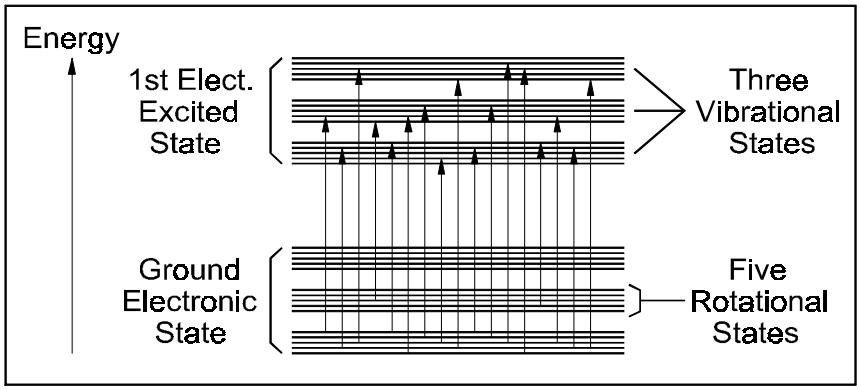

Figure 4-6

As for the atomic case the vertical arrows represent allowed transitions, but in this case *only one electronic transition* is involved whereas Figure 4-4 showed four electronic transitions. Most of the arrows are shown originating in the lowest vibrational state since most molecules are in this state at room temperature. Thus each electronic transition consists of not one (as in the case of atoms) but many closely spaced transitions or lines involving different vibrational and rotational states. For diatomic molecules in the gas state all of these different lines can be resolved (in many cases). However, for larger molecules in solution these lines are so closely spaced and broadened due to intermolecular interactions that resolution is impossible. This causes the absorption spectrum to take the form of a broad band as shown in Figure 4-7.

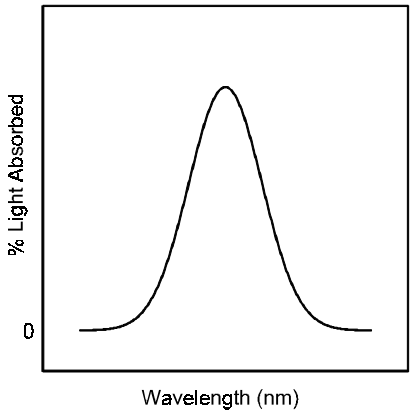

Figure 4-7

For the purposes of this text the difference between atomic and molecular absorption spectra is in the width of the absorption line or band - atomic lines are on the order of 10^{-3} nm wide whereas molecular bands are about 10^{2} nm wide.

There are several different types of electronic transitions which give rise to the absorption of light in the visible and uv portions of the spectrum. Only two will be mentioned here. For transition metal ions often the d electrons are involved in the transitions (called d-d transitions). This is the reason that transition metal ions with unfilled d shells are often coloured in solution. For organic molecules the bonding electrons often absorb the light energy. For single bonds (sigma bonds) the absorption of light promotes an electron from a bonding orbital to an antibonding orbital (called a $\sigma \rightarrow \sigma^*$ transition) and often results in the dissociation of the molecule, an obviously undesirable occurrence. For a molecule with a double bond, a pi bonding electron absorbs the energy (called a $\pi \rightarrow \pi^*$ transition) and the molecule

does not dissociate, since a sigma bond still holds the molecule together. Thus visible-uv spectroscopy is very often used for molecules that are unsaturated, often aromatic molecules.

One question that might be asked is "What happens to the energy of the photon after absorption?" Very often this is degraded to heat after many collisions in the solution. Sometimes this energy is removed in other ways - more will said on this topic later.

So far only the absorption of light has been discussed. Light can also be emitted by molecules and atoms. This is just the reverse of the process which has been explained above. Note, however, that to emit light an atom or molecule must somehow first be placed in an excited state, and this is not always a simple task. Methods of achieving this, and the emission of light in general, will also be discussed later.

4.2 ABSORPTION SPECTROSCOPY IN SOLUTION

Since atoms do not normally exist in solution, this discussion will pertain to ions and molecules, in other words the absorption bands will be broad. The majority of work done with uv-vis spectroscopy involves quantitative analysis, although sometimes this type of spectroscopy is done for qualitative purposes. Thus a relationship between the concentration of absorbing species and the amount of light absorbed needs to be developed.

4.2.1 THE BEER-LAMBERT LAW

The Beer-Lambert law relates the amount of light absorbed to the concentration and dimensions of the absorber. It is often simply called Beer's law. Note that, while considering the derivation to follow, the absorbing solution is often placed in a square cell (called a cuvette) made of glass or quartz which usually has a cavity 1 cm in length as shown in Figure 4-8.

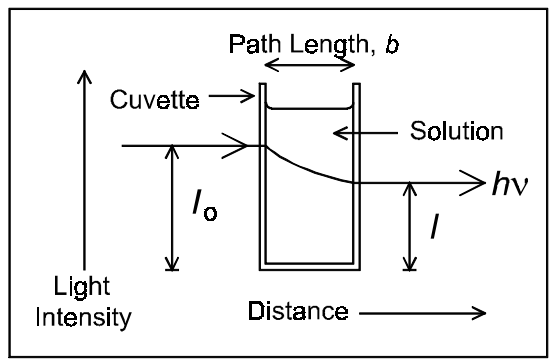

Figure 4-8

Let I_o be the intensity of light entering the solution (in photons/cm^2·s), I be the intensity leaving the solution (I is also used for intensity in general), x be distance (in cm), b be the optical path length through the solution (in cm), c be the concentration of absorber (in mol/L), P be the probability that on collision with a photon a molecule will absorb that photon (in molecules^{-1}), and σ be the molecular cross section (in cm^2) - this can be considered the molecular area effective in absorbing light. NOTE - most textbooks use the symbol "P" to represent light power (in J/cm^2·s) in the derivation of Beer's law. Probability and power are two entirely different quantities and should not be confused. Beer's law can be derived using either light power or light intensity. Intensity will be used here.

Imagine a very thin slice of solution, dx, so thin that intensity, I, can be considered constant across it. The number of molecules in this slice (per square centimetre), M, is

$$M = (0.001 \text{ L/cm}^3)Ncdx \qquad (4\text{-}5)$$

where N is Avogadro's number ($6.02\text{x}10^{23}$ molecules/mol) and the number 0.001 is simply a conversion factor between litres and cubic centimetres. The number of photons absorbed per second, which is the same as the loss in light intensity, $-dI$, is

$$-dI = MI\sigma P = 0.001NP\sigma Icdx \qquad (4\text{-}6)$$

This differential equation can be solved by separation of the variables, followed by integration.

$$-\frac{dI}{I} = 0.001NP\sigma c dx \tag{4-7}$$

Integration is carried out from $x = 0$, where $I = I_o$, to $x = b$, where $I = I$.

$$-\int_{Io}^{I}\frac{dI}{I} = 0.001NP\sigma c\int_{0}^{b} dx \tag{4-8}$$

The result of integration (Beer's law) is

$$\ln\left(\frac{I_0}{I}\right) = 0.001NP\sigma bc \tag{4-9}$$

This is usually written

$$A = \varepsilon bc \tag{4-10}$$

where A is the *absorbance* and equals $\log(I_o/I)$ (note the change from base e to base 10) and ε is the *molar absorptivity* and has the units L/mol·cm. In terms of the quantities used above $\varepsilon = 0.000434NP\sigma$. This includes the factor 0.434 which converts from base e to base 10.

Beer's law indicates that absorbance is directly proportional to concentration, suggesting that measurement of absorbance should be an excellent method for performing quantitative analysis. It also indicates that absorption of light is *not linear* in either concentration or path length, a result that is not necessarily intuitive.

Note that in deriving Beer's law P, σ, and c have been assumed to be constant. c can certainly be considered constant (*in a given solution*) and probably also σ. However, P will depend strongly on wavelength, and to some extent on solution refractive index and solution concentration. More will be said about this later.

4.2.2 PHOTOMETRIC INSTRUMENTATION

The next step in the discussion is to examine how absorbance is measured. This is done with an instrument called a *spectrophotometer*. A block diagram of this instrument is shown in Figure 4-9.A few words should be said about each component of the spectrophotometer.

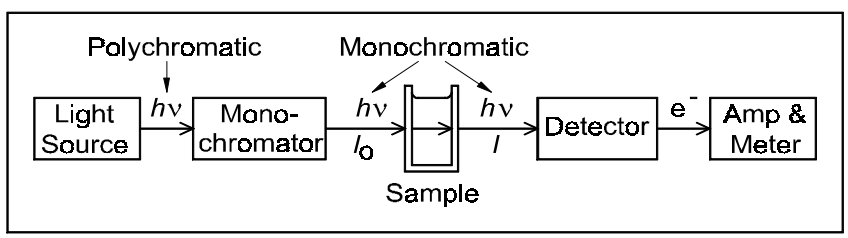

Figure 4-9

Light source

If measurements are to be made only in the visible region, then the light source usually used is an ordinary tungsten filament incandescent lamp. These lamps emit wavelengths at useful intensities from the infrared (well above 700 nm) to about 350 nm.

If wavelengths below 350 nm are needed, a glowing wire cannot be used since all known substances melt at temperatures high enough to provide the needed energies. An entirely different method of obtaining light must be employed. The method employed usually involves the use of an electric discharge

in a tube partially filled with a specific gas. In spectrophotometers this gas is usually hydrogen, H_2, or deuterium, D_2. A diagram of such a gas-discharge tube is shown in Figure 4-10.

In this tube electrons are "boiled" off the heated cathode and travel across the tube to the anode. Since several hundred volts are usually applied between the cathode and anode, these electrons can acquire a high energy before striking a gas molecule. Such a collision can result in excitation of the gas molecule.

$$e^- \text{ (high energy)} + H_2 \rightarrow H_2^* + e^- \text{ (lower energy)}$$

The excited gas molecule, represented by H_2^*, can then decay to the ground state or a lower excited state by emitting a photon of light, represented by $h\nu$.

$$H_2^* \rightarrow H_2 + h\nu$$

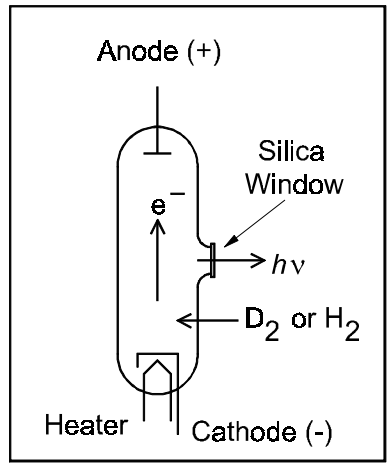

Figure 4-10

This emitted light can be of very high energy (low wavelength), depending on the gas used in the tube. Fluorescent lights work on the same principle, except that the gas used is mercury, not hydrogen, and the tube is coated with a substance (a phosphor) to convert the short wavelength ultraviolet light given off by the gas discharge to visible light.

Monochromator

Unfortunately almost all light sources used in spectrophotometers emit polychromatic light. This is light that contains a broad range of wavelengths. For best results, the light used should be monochromatic (a single wavelength or a small range of wavelengths). The device that selects a "single" wavelength from polychromatic light is called a monochromator. There are several methods which can be employed to do this. A filter can be used to pass a certain colour (a small band of wavelengths). Filters are inexpensive, but usually pass a wide band of wavelengths and are inflexible since the band of wavelengths cannot be varied (the wavelength cannot be changed). Prisms and diffraction gratings can be used to break white light into its individual wavelengths and then any one of these wavelengths can be selected. Modern instruments almost universally use diffraction gratings - these are mirrors with closely spaced fine lines scribed on them. Compact disks behave in a similar fashion to gratings, and show the effect of breaking white light into its components. A simplified diagram of a grating monochromator is shown in Figure 4-11.

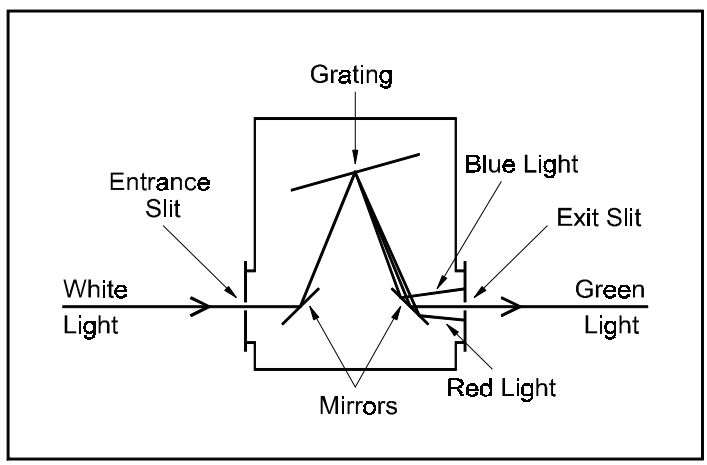

Figure 4-11

The position of the grating is varied to select the wavelength desired for the analysis.

Sample cell

The sample cell is usually a square container (see Figure 4-8) with a path length of 1.00 cm made of glass or silica (SiO_2). Glass, which contains materials which absorb light below 340 nm, is used in the visible and silica (or quartz), which transmits down to 190 nm, is used in the ultraviolet. Silica could also be used in the visible, but is much more expensive and therefore only used when needed. Some instruments use flow cells which are never removed from the instrument. Such cells, shown diagramatically in Figure 4-12, also normally have path lengths of 1.00 cm. There are certain advantages to the use of flow cells: (a) the cell is never moved and thus is not subject to errors caused by changes in its position, (b) only one cell is ever used, so matched cells are not necessary, (c) the cell is not handled and thus the windows do not get dirty (on the outside), and (d) the measurements can be done very rapidly since cells do not have to be manipulated. However, a pump must be available if a flow cell is used.

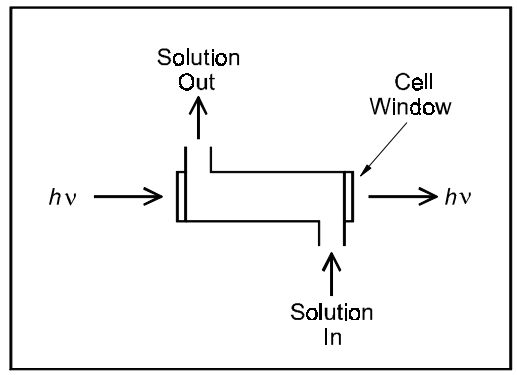

Figure 4-12

Detector

The light signal from the sample must be converted to a form which can somehow be displayed. Display is done with an electronic device, *e.g.* a meter, and thus the detector must convert the optical signal (beam of photons) into an electronic signal (an electric current). There are several types of devices that accomplish this. Perhaps the simplest, and one often used in simple spectrophotometers is a vacuum photodiode. This is simply an evacuated glass or silica container (depending on whether the light to be detected is visible or uv) which contains two electrodes (thus the name diode), see Figure 4-13. The cathode or negative electrode is a large semicircular metal foil coated with a special material with a low work function. This means that electrons can be easily removed from this surface coating. A photon to be detected strikes the cathode and ejects an electron (providing the photon has enough energy to do this). This electron is attracted to the anode, which is usually a thin wire. Thus a current flows through the diode when light strikes the cathode. Since each photon produces an electron, the electric current is directly proportional to the light intensity striking the detector. Actually not every photon produces an electron, but a constant fraction of photons produces electrons, still maintaining the proportionality between intensity and current. This proportionality makes photometric measurements much simpler.

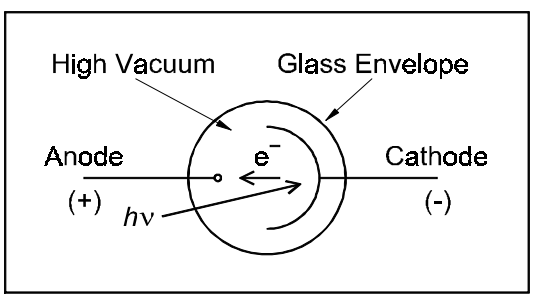

Figure 4-13

Amplifier and meter

The amplifier is an electronic device that amplifies the very small currents produced in the detector and makes them capable of being displayed on the various display devices used. The meter is one means of displaying the amplified current from the detector. Thus the meter reading will be directly proportional to the light intensity exiting the cell. As long as the intensity of the light source does not

vary with time, any changes in the meter reading will directly reflect changes in the absorption of light in the cell.

4.2.3 SPECTROPHOTOMETRIC MEASUREMENTS

Now that the instrument used to make spectrophotometric measurements has been examined, consideration must be given to how the measurements are made and what is being measured. It is obvious why the instrument contains a light source, sample compartment, detector, and amplifier. However, why is it necessary to have a monochromator? The probability that a photon will be absorbed, *P*, is a strong function of wavelength, λ. This gives rise to an absorption band that is usually bell shaped. There are wavelengths at which *P* is zero, $\lambda < \lambda_1$ and $\lambda > \lambda_2$ in Figure 4-14. Any light at these wavelengths which falls on the sample is not absorbed at all. *P* is maximum at λ_{max}, and thus absorbance is also maximum at λ_{max}. Thus if white light is passed through the sample, much of the light will always be transmitted, and the sensitivity will be low. Sensitivity will be maximum if light only at λ_{max} is selected and passed through the sample cell. For this reason, and others to be discussed later, the monochromator is an essential part of the spectrophotometer.

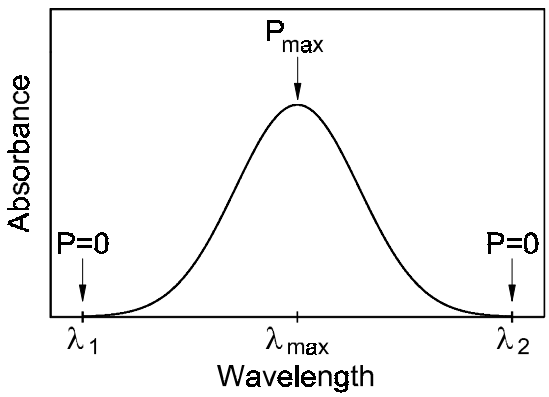

Figure 4-14

The first step in the use of a spectrophotometer is to set the wavelength selected by the monochromator to λ_{max}. The next step is to properly zero the instrument. One might expect that if the light beam were completely blocked, that the meter would read zero (no photons, no detector current). Unfortunately a small current still flows in the detector circuit even with no light falling on the detector. This current is called the *dark current*. It must be balanced out, so many instruments have a control associated with the amplifier, called the *dark current control*, or *zero*, that adjusts the meter to zero when the light beam is totally blocked. Thus after adjusting the monochromator, the light beam is blocked and the meter set to zero.

The next step is to place a cell in the light path (with the light beam on). This cell should contain everything in the sample solution *except* the analyte and is called the blank. Under these conditions, for example, say that the meter reads 78.6. Note that the instrument reading is proportional to light intensity. Thus it can stated that I_o is equivalent to a reading of 78.6. Why is the blank necessary, why not just leave the cell out of the beam when establishing I_o? There are two reasons for this:

1) The sample may contain a material that absorbs light in addition to the unknown. If this same material is in the blank, then its absorbance is cancelled out and only the unknown absorbance is measured.

2) The sample cuvette itself reflects a small amount of light (5 to 10%), as shown in Figures 4-15 and 4-16, and this must be compensated.

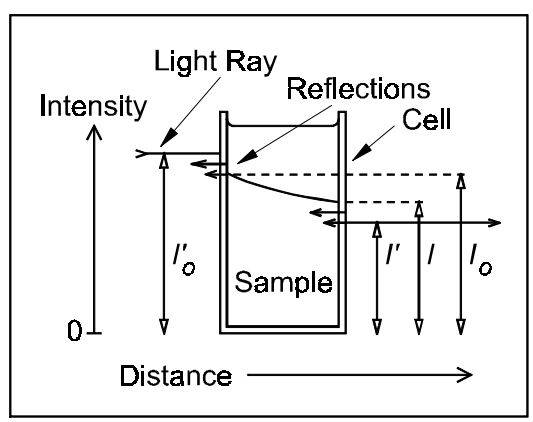

Figure 4-15

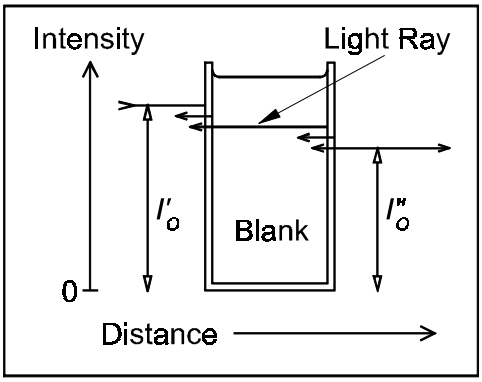

Figure 4-16

Referring to Figure 4-15, the light exiting the monochromator is I_o' and that exiting the cuvette is I'. However, the light incident on the sample solution is I_o and that leaving the solution is I. I_o is less than I_o' and I' is less than I because of reflections at the walls of the cuvette. The true absorbance of the solution is $\log(I_o/I)$. However, if measurements were made without a blank solution, I_o' and I' would be the intensities measured and absorbance would be calculated as $\log(I_o''/I')$, resulting in an error in which the measured absorbance was always greater than the true absorbance.

Referring to Figure 4-16, when a blank is placed in the light beam, the light exiting the sample cell is I_o''. This is taken to represent the light striking the sample solution, and the light exiting the cell when it contains the sample, I', (Figure 4-15) is taken to represent the light exiting the solution. Thus absorbance is calculated as $\log(I_o''/I')$. This does not strictly measure I_o and I, but since $I_o''/I' = I_o/I$, the ratio is correct, and thus the absorbance calculated is also correct. In this way the reflectance of the sample cell is corrected.

The final step in the measurement is to place a cell containing the unknown in the instrument and take a reading. Say that this reading is 35.4. Thus intensity I is equivalent to 35.4. Absorbance can now be calculated.

$$A = \log(I_o/I) = \log(78.6/35.4) = 0.346$$

Note that the instrument does not read directly in absorbance, it provides two intensities which must be combined to give absorbance.

The ratio, I/I_o, is called the *transmittance* and given the symbol T. Thus

$$T = I/I_o \quad \text{(normally less than unity)} \qquad (4\text{-}11)$$

and

$$A = \log(I_o/I) = \log(1/T) = -\log(T) \qquad (4\text{-}12)$$

The normal procedure with spectrophotometers is to employ a meter that has 100 scale divisions. A second control, which changes the amplification factor of the amplifier, is used when the blank is placed in the instrument to adjust the meter to read full scale (100). Thus in the example above with the blank in place, the meter is adjusted from 78.6 to 100 with the *100 %T* control. This would cause the sample to read 45.0 instead of 35.4, since the increase in gain would affect both measurements equally. This does not affect either A or T, since

$$T = 35.4/78.6 = 45.0/100.0 = 0.450$$

It is now convenient to introduce the concept of *percent transmittance*.

$$\%T = 100T \qquad (4\text{-}13)$$

In the example above, the *%T* is 45.0. Note that by adjusting the blank reading to 100, the sample reading (45.0) will automatically be in *%T*. Thus when set up in the fashion described above, the instrument directly reads the percentage of light transmitted through the sample as compared to the blank. The absorbance is easily calculated from *%T* as follows:

$$\%T = 100I/I_o \qquad (4\text{-}14)$$

or $$I_o/I = 100/\%T \quad (4\text{-}15)$$

$$A = \log(I_o/I) = \log(100/\%T) = 2 - \log(\%T) \quad (4\text{-}16)$$

Many simple spectrophotometers have meters with both *A* and *%T* scales. Since the *A* scale is nonlinear, it is best to measure *%T* from the linear scale and convert mathematically to *A*. Modern instruments often contain computers that calculate absorbance and that perform some of the manual operations described above automatically without operator intervention.

4.2.4 SPECTROPHOTOMETRY IN QUANTITATIVE ANALYSIS

The instrumentation and method for making a measurement with a spectrophotometer have been described. The next topic to be discussed is how these measurements are used in quantitative analysis. There are several methods of doing quantification.

Absolute method

The simplest method for quantitative analysis would be to assume that Beer's law is valid, measure *%T*, convert to *A*, look up the value of the molar absorptivity, ε, in a table, and calculate concentration, $c = A/\varepsilon b$. This is known as the "absolute" method.

For example, the molar absorptivity of a Lewis base complex of Cu(II) is 24,300 L/mol·cm. 25.0 mL of sample are mixed with 25.0 mL of the complexing reagent and the *%T* of the resulting solution is measured as 46.8% in a 1.00 cm cuvette. What is the copper concentration in the original sample?

$A = 2 - \log\%T = 2.000 - 1.670 = 0.330$

$c = A/\varepsilon b = 0.330/(2.43\text{x}10^4\ \text{L/(mol)cm})(1.00\ \text{cm}) = 1.36\text{x}10^{-5}\ \text{mol/L}$

This is the concentration in the diluted solution. For the original sample,

$c = (1.36\text{x}10^{-5}\ \text{mol/L})(50.0\ \text{mL})/25.0\ \text{mL} = 2.71\text{x}10^{-5}\ \text{mol/L}$

This method is almost never used. There are several reasons for this: (a) there may be no published value of ε, (b) there may be some doubt about the "accuracy" of the published value of ε, (c) the conditions employed when the *%T* of the unknown was determined may not have been the same as those used when ε was measured, and (d) Beer's law may not be strictly valid under the conditions used in the experiment. This last problem needs closer examination.

Deviations from Beer's law

1) Real Deviations: If, for example, the refractive index of the solutions changes from one measurement to another, Beer's law is invalid. Such deviations are not usually problems at the concentrations encountered in spectrophotometric measurements.

2) Chemical Deviations: These occur when the coloured material is involved in a chemical equilibrium which is affected by concentration and which is unknown to the analyst. An example is probably the best way to illustrate this effect.

Assume that the conjugate base of a Brønsted acid is coloured (the acid is colourless) and is being determined spectrophotometrically. Phenolphthalein could be such an acid-base system. Also assume

that $\varepsilon = 10^3$ L/mol·cm, $b = 1$ cm, $K_b = 1x10^{-5}$, and that the solution is unbuffered. The chemical equilibrium which causes the apparent deviation from Beer's law is

$$A^- + H_2O \rightarrow HA + OH^-$$

The only coloured species in this equilibrium is A^-. If one can assume that $[OH^-] >> [H^+]$, then it can be shown that $[A^-] = F_b - [OH^-]$. It will also be assumed that $[OH^-] = (-K_b+\{K_b^2+4K_bF_b\}^{1/2})/2$. With these equations a table (Table 4-3) of concentrations and absorbances can be made for different formalities of the base.

Table 4-3

Formality of base	$[A^-]$	Absorbance	Slope(ΔAbs/ΔF)
$1.00x10^{-4}$	$7.30x10^{-5}$	0.073	844
$2.00x10^{-4}$	$1.60x10^{-4}$	0.160	889
$4.00x10^{-4}$	$3.42x10^{-4}$	0.342	921
$7.00x10^{-4}$	$6.21x10^{-4}$	0.621	940
$1.00x10^{-3}$	$9.05x10^{-4}$	0.905	950
$1.2x10^{-3}$	$1.095x10^{-3}$	1.095	954

If the analyst were unaware of the chemical equilibrium involved, she/he would assume that $[A^-]$ was equal to formality and would plot absorbance *vs* formality rather than absorbance *vs* $[A^-]$ (which would be unknown). Such a plot is shown in Figure 4-17. In this plot the actual data are represented by a solid line and the tangent to the data at the intercept by a dashed line. Absorbance increases more rapidly than formality (on a relative basis) and the plot is not linear, thus seeming to indicate that Beer's law is not obeyed. Since the slope increases with increasing formality (see Table 4-3) this type of behaviour produces a positive deviation from Beer's law. Positive deviations are normally due to chemical problems. If the chemist had been aware of the problem, the plot would have been made of absorbance *vs* $[A^-]$. This plot would have been linear, indicating that Beer's law was indeed valid. When investigating a spectrophotometric method for the first time, the chemist should always be very careful to determine whether any "chemical deviations" from Beer's law are observed.

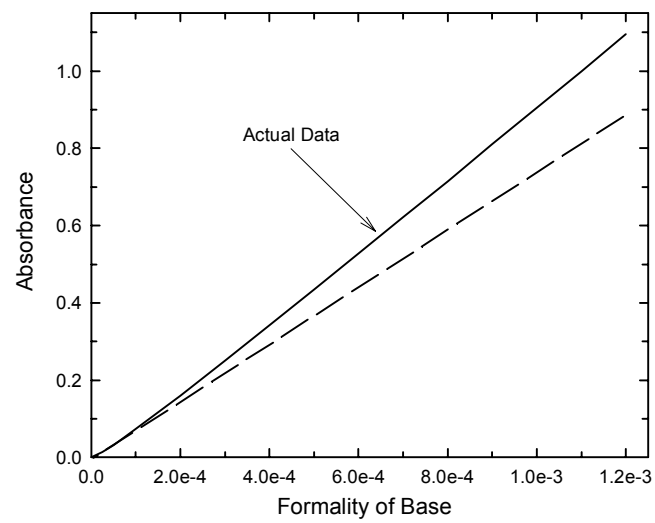

Figure 4-17

3) Instrumental Deviations: There are two major types of such apparent deviations from Beer's law, those due to the inability of the monochromator to provide light of a single wavelength and those due to stray light in the monochromator.

a) Non-monochromaticity of light. Recall that *P*, or ε, is a function of λ, and that Beer's law was derived assuming that *P* was constant. Thus Beer's law is strictly valid for only a single wavelength of light. If the monochromator produces only a single wavelength, there will be no problem. However, no

monochromator, no matter how good, can produce only a single wavelength, and the ones used in simple spectrophotometers usually produce a fairly broad band of wavelengths, ranging from a width of 10 to 20 nm at half the band maximum.

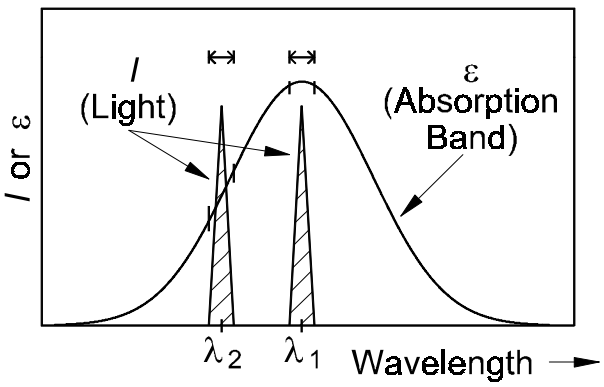

Figure 4-18

This situation is depicted in Figure 4-18. In this figure molar absorptivity, ε, and intensity of light from the monochromator, *I*, are both plotted on the *Y* axis. The band of light from the monochromator is represented as triangular in shape, a reasonable approximation to the actual behaviour. The wavelength value set on the instrument is the wavelength at the peak of the triangle and the bandwidth listed by the manufacturer is the width of the triangle at half the peak height. At wavelength one, the molar absorptivity does not change rapidly with wavelength and the fact that the monochromator produces a small band of wavelengths causes no problem, since the molar absorptivity is approximately the same for all wavelengths exiting the monochromator. This is normally the case at the band maximum. However, at wavelength two, ε varies considerably with λ, and Beer's law will not be obeyed. Thus there are several advantages to setting the monochromator at the wavelength of maximum absorption - it gives maximum sensitivity and best adherence to Beer's law, and small variations in the setting from day to day will have a minimum effect.

This type of deviation from Beer's law will always produce negative deviations. This situation is depicted in Figure 4-19.

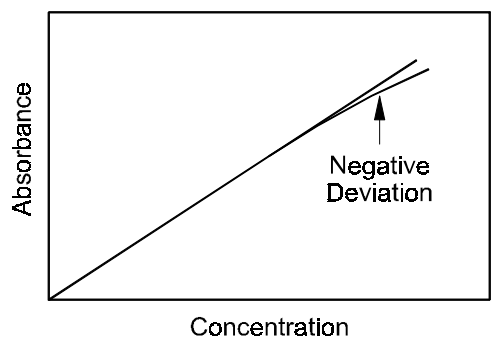

Figure 4-19

b) Stray light. In the case just discussed above, it was pointed out that a monochromator produces not just a single wavelength, but a band of adjacent wavelengths. This is an inherent limitation of monochromators and cannot be avoided. Monochromators also pass some light that is not in this narrow band, and may be far outside the band. This light "should not be there" and could, in theory, be eliminated by careful design. This type of light is called stray light. It causes the same sort of problem mentioned above, but usually to a larger extent.

Assume that the monochromator passes 1% stray light at wavelengths that are not absorbed by the sample at all. Thus 1% of the total light will always come through the solution, no matter how concentrated the sample. Thus the maximum absorbance which can be measured will be $A_{max} = \log(100/1) = 2.00$, regardless of concentration. This also produces a negative deviation from Beer's law. Stray light usually causes deviations to appear above absorbances of between 1 and 1.5 for inexpensive instruments and is one reason that attempts are made to keep measurements below absorbances of one.

Use of a calibration curve

Since Beer's law cannot always be trusted, one normally prepares a calibration curve with a set of standards which are as similar to the unknown as possible. The first point on this curve ($A = 0$ at $c = 0$) is determined by the blank. This solution normally contains all reagents used in the analysis but no

analyte and is not simply distilled water. The remaining points are determined from the standards containing known concentrations of analyte. The absorbance of the unknown is then measured and its concentration is read from the calibration graph. This method has all the advantages and disadvantages outlined in Chapter 3 (see Figure 3-3 for an example of a spectrophotometric calibration curve). One main advantage is that it provides good results even if deviations from Beer's law occur (see Figure 4-20).

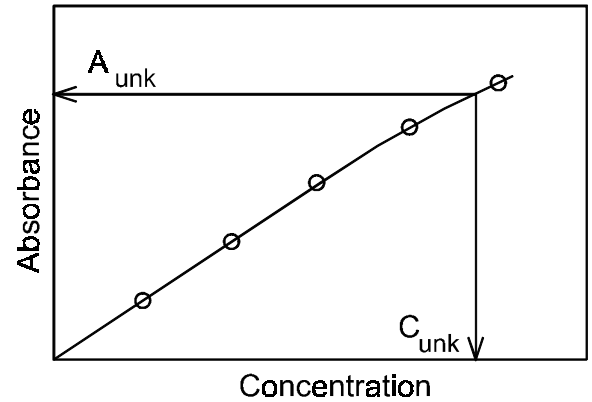

Figure 4-20

A set of standards containing 2.00, 4.00, 6.00, and 8.00×10^{-5} M Co(II) and a colour forming reagent gave a set of absorbance data which, when fit by the method of linear least squares, produced the following values for the calibration curve: slope = 1.344×10^4, intercept = 0.0015. If a sample produces an absorbance reading of 0.377, what is the concentration of Co(II) in the sample?

The calibration curve has the form

$A = 0.0015 + 1.344 \times 10^4 c$ or

$c = (A - 0.0015)/1.344 \times 10^4 = (0.337 - 0.0015)/1.344 \times 10^4 = 2.50 \times 10^{-5}$ mol/L

In theory the intercept above should be zero. However, indeterminate error in the data points usually gives rise to a small, but finite intercept.

Standard addition

The method of standard addition, as explained in Chapter 3, can also be employed in spectrophotometry. It is especially useful in cases where certain matrix effects must be dealt with. It should be kept in mind that this method requires that the instrumental response be linear in analyte concentration. Thus Beer's law must be obeyed if standard addition is to be used.

The absorbance of a solution containing an unknown amount of Cu(II) and an excess of sodium diethyldithiocarbamate (a colour-forming reagent) is measured as 0.395. 1.00 mL of a 5.00 ppm Cu(II) standard is added to 10.00 mL of the sample. The absorbance of this solution is measured as 0.486. What is the Cu(II) concentration in the sample?

$0.395 = \varepsilon b c_u$

$0.486 = \varepsilon b\{c_u(10.00 \text{ mL})/(11.00 \text{ mL}) + (5.00 \text{ ppm})(1.00 \text{ mL})/(11.00 \text{ mL})\}$

$0.486/0.395 = (10.00/11.00) + 5.00 \text{ ppm}/11.0(c_u)$

$c_u = 5.00 \text{ ppm}/(11.00)(0.486/0.395 - 10.00/11.00) = 1.41 \text{ ppm}$

Concentration range and accuracy.

The questions of concentration range and accuracy in spectrophotometry will now be examined. First the range - what values of concentration are normally measured by this technique? In spectrophotometry (in the visible and uv) ε is normally between 10^3 and 2.5×10^4, *b* is normally 1 cm, but is sometimes made 10 cm, and *A* usually falls between 0.1 and 1.0. Using the minimum and maximum values above in Beer's law, the concentrations normally measured fall between about 10^{-3} and 4×10^{-7}M. Thus spectrophotometry is considered a trace technique; it is not used to measure the main components in a sample (those making up 1% or more of the sample).

What is the accuracy that one might expect from this technique? The approach taken to answer this question is not specific to spectroscopy but is quite general. One applies differential calculus to the relationship between response and concentration to determine what small change in concentration will result from a small change in the response. In spectrophotometry Beer's law is employed to determine the small change (or error) in concentration, *dc*, that is caused by a small change (or error) in transmittance, *dT*. From Beer's law written in terms of transmittance

$$-\log(T) = \varepsilon bc \tag{4-12}$$

or

$$-\ln(T) = 2.303\varepsilon bc \tag{4-17}$$

after differentiation

$$d(-\ln T) = 2.303\varepsilon b dc \tag{4-18}$$

or

$$\frac{-dT}{T} = 2.303\varepsilon b dc \tag{4-19}$$

Since one is usually interested in the relative error in *c*, *dc/c*, both sides of Eqn. (4-19) are divided by c.

$$\frac{-dT}{cT} = 2.303\varepsilon b\left(\frac{dc}{c}\right) \tag{4-20}$$

Solve (4-17) for *c* and substitute on the left hand side of (4-20).

$$\frac{-dT}{T}\left(\frac{-2.303\varepsilon b}{\ln T}\right) = 2.303\varepsilon b\left(\frac{dc}{c}\right) \tag{4-21}$$

Since $2.303\varepsilon b$ cancels on both sides of (4-21) the result is

$$\frac{dc}{c} = \frac{dT}{T\ln T} \tag{4-22}$$

where *dc/c* is the *relative error* in *c* and *dT* is the *absolute error* in *T*.

Several different types of errors can arise in spectrophotometry. Only two of these types of errors will be considered here.

1) Errors that are independent of *T*: In this case *dT* is constant. Errors in reading the meter (or errors in the meter itself) and fluctuations in the amplifier signal (amplifier noise) fall into this category. In this case $dT = K_1$ and

$$\text{rel error in } c = dc/c = K_1/T\ln T \tag{4-23}$$

It can be readily shown by the methods of calculus that, if these are the only types of errors of any magnitude, the relative error in *c* is minimum when $T = 0.368$ ($A = 0.434$). If, for example, $dT = K_1 = 0.005$

(a 0.5% error in T), then the minimum error in c will be about 1.4% (at $T = 0.368$) and the error in c will be less than 2% between T of 0.12 and 0.70 ($A = 0.92$ and 0.16). In this case one would attempt to keep one's measurements between absorbances of about 0.1 and 1 in order to keep the error in c small. The relative error in c becomes very large at either very low or very high absorbances.

2) Errors that are directly proportional to T: In this case $dT = K_2T$. Such errors would arise through fluctuations in lamp intensity or variations in the cell position (or dirty cells). Note that here the *relative error* in T, dT/T, is constant.

$$\text{rel error in } c = dc/c = K_2/\ln T \tag{4-24}$$

If this type of error predominates, then the relative error in c decreases as T decreases (A increases). For example, if $K_2 = 0.005$, the error in c will be 2% at $T = 0.78$ ($A = 0.11$), 0.2% at $T = 0.10$ ($A = 1$), and 0.11% at $T = 0.01$ ($A = 2$).

Figure 4-21 demonstrates how these errors vary with T. The plot with the open circles represents the behaviour of constant errors (Type 1) in which $dT = 0.01$. Note that the relative error in c increases rapidly at both high and low T (low and high A, low and high concentration). The plot with open squares represents the behaviour of errors proportional to T (Type 2) in which $dT/T = 0.01$. In this case the relative error in concentration is high only at high T (low A or low concentration).

The more usual case would be some combination of these two types of errors, $dT = K_1 + K_2T$. If these errors are not random, but always in one direction, then

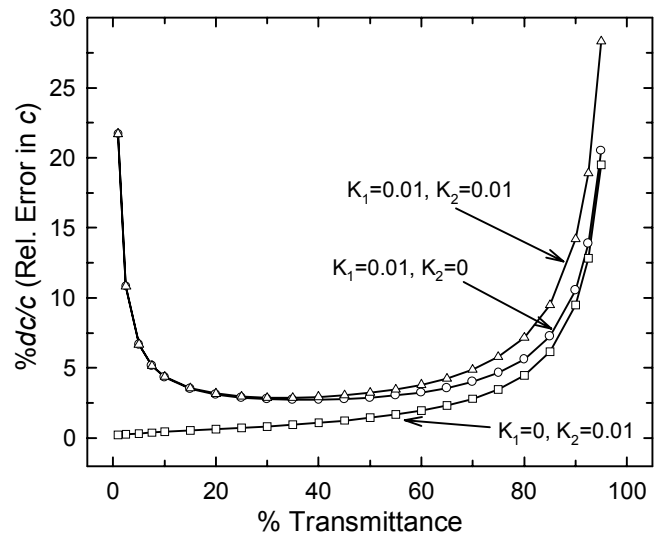

Figure 4-21

$$\text{rel error in } c = dc/c = K_1/T\ln T + K_2/\ln T \tag{4-25}$$

However, if these errors are unrelated and random which is more likely, then

$$\text{rel error in } c = \{(K_1/T\ln T)^2 + (K_2/\ln T)^2\}^{1/2} \tag{4-26}$$

In either case the error will be a minimum at some value of T which will depend on both K_1 and K_2. The plot with open triangles in Figure 4-21 represents the case of random errors in which both types of errors are present at the 1% level. Both types of errors affect the total error at high T (low concentration), but only the errors independent of T affect the total error at low T (high concentration). In this case the minimum relative error in concentration is about 2.9% and occurs at a T of about 0.35. The total error is below 5% between T values of 0.08 ($A = 1.10$) and 0.70 ($A = 0.15$), again indicating that it would be wise to limit measurements to absorbances between 0.1 and 1.0 if possible. Regardless of the type of error present in the measurement, the relative error in concentration will always be high when measurements are made at low absorbance values.

In summary, spectrophotometry is normally used in trace analysis (concentrations less than 10^{-3}M) with accuracies approaching 1% when care is taken. Contrast this with titrations which are normally used for major component analysis at accuracies approaching 0.1%. Thus these different techniques are complimentary.

A sample produces an absorbance reading of 0.053 on a spectrophotometer known to have errors of both types. Those that are independent of T are believed to be at about the 0.5% level and those proportional to T are at the 0.7% level. What is the expected relative error in concentration for the coloured unknown in the sample if the measurement errors are independent of each other and random? Since the errors are random and independent, use Eqn.(4-26).

$K_1 = 0.005$ and $K_2 = 0.007$

$-\log T = A = 0.053$ or $T = 10^{-0.053} = 0.885$

rel. error in $c = \{[0.005/0.885(\ln 0.885)]^2 + [0.007/\ln 0.885]^2\}^{1/2}$

rel. error in $c = \{(-0.0463)^2 + (-0.0574)^2\}^{1/2} = 0.0737$

rel. error in $c = 7.4\%$

Multi-colour analysis

What happens when a solution contains more than one coloured analyte? If the absorption bands of the analytes do not overlap (interfere), then each can be analyzed individually from separate calibration curves as if the other analytes were not present. If a background colour exists in the sample which can be duplicated in the blank, then this background subtracts out and does not affect the measurement. However, if two or more analytes with overlapping spectra exist in the sample, the situation becomes more complicated.

Individual absorbances are additive and, if Beer's law holds, the problem is readily solved. For a multi-component mixture (containing components X, Y, *etc*.)

$$A = \log(I_o/I) = A_x + A_y + \ldots \quad (4\text{-}27)$$

or

$$A = \varepsilon_x bc_x + \varepsilon_y bc_y + \ldots \quad (4\text{-}28)$$

The individual spectra of a two component mixture and the combined spectrum of the two are shown in Figure 4-22. It is only the combined spectrum which can be measured by the spectrophotometer. One must choose as many wavelengths for measurements as there are analytes in the mixture. These wavelengths are chosen such that the contribution to the absorbance from one component at a particular wavelength is large while the contributions from the other components are small. For a two component mixture, measurements must be made at two different wavelengths. In this case one might choose λ_1 and λ_2 as shown in Figure 4-22. The molar absorptivities for all the different components must be known at all measurement wavelengths. For a two component mixture ε_{1X}, ε_{1Y}, ε_{2X}, and ε_{2Y} must be known. Then the solution absorbances are measured at all selected wavelengths and a set of simultaneous, linear equations is set up and solved for the analyte concentrations. There

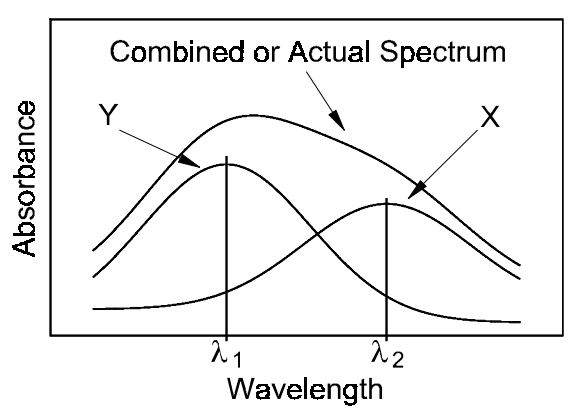

Figure 4-22

will be as many equations as there are measurement wavelengths and analytes. For a two component mixture

$$A_1 = \varepsilon_{1X} b[X] + \varepsilon_{1Y} b[Y] \qquad \text{at } \lambda_1 \qquad (4\text{-}29)$$

$$A_2 = \varepsilon_{2X} b[X] + \varepsilon_{2Y} b[Y] \qquad \text{at } \lambda_2 \qquad (4\text{-}30)$$

The above equations are solved for [X] and [Y]. Note that Beer's law must be valid in order to get correct results from this method.

The following data are available: for substance M, ε_1 = 3000, ε_2 = 10,000, and for substance N, ε_1 = 12,000 and ε_2 = 4000, all dimensions are L/mol·cm. The %*T*s of a mixture of M and N are 10.0% and 18.3% at λ_1 and λ_2 respectively. What are the concentrations of M and N in the sample, assuming a cell path length of 1.00 cm?

A = 2 - log(10.0) = 1.000 at λ_1

A = 2 - log(18.3) = 0.737 at λ_2

(1) 1.000 = 3000(1.00)[M] + 12000(1.00)[N]

(2) 0.737 = 10000(1.00)[M] + 4000(1.00)[N]

From equation (2)

[M] = (0.737 - 4000[N])/10000 = 7.37×10^{-5} - 0.4000[N]

Substitute this into equation (1)

1.000 = 3000(7.37×10^{-5} - 0.4000[N]) + 12000[N]

[N] = (1.000 - 0.221)/(12000 - 1200) = 7.21×10^{-5} M

Substitute this result into equation (2)

0.737 = 10000[M] + (4000)(7.21×10^{-5})

[M] = (0.737 - 0.288)/10000 = 4.49×10^{-5} M

Spectrophotometric titrations

Any instrument that responds to concentration can be used to follow a titration, and spectrophotometers are no exception. These can be used in two ways. In the first one would add a coloured indicator and follow the indicator colour change with the instrument. In this case the instrument substitutes for the eye in detecting the rapid change in colour at the end point. This method is useful in automated titrations. Since the theory is much the same for visual observation and instrumental observation, this will be discussed no further.

The second method is to use a spectrophotometer to follow a titration in which one or more of the reagents is coloured. In this case no indicator is added or needed. Since $A = \varepsilon bc$ (assuming Beer's law is valid), the calculated response is linear in concentration. This gives rise to a type of titration referred

to as a *linear titration*. These are quite general and are observed with many instrumental techniques. Here spectrophotometry will be used as an example of such a titration.

Assume a general titration reaction of the following type

$$T + U \rightarrow P_1 + P_2$$

where T represents the titrant, U the unknown, and P_1 and P_2 the products. Also assume that *only* U is coloured. The results derived below apply only to a 1:1 reaction, but other types of reactions can be treated in the same manner.

Before the equivalence point:

$$A = \varepsilon_u b[\mathrm{U}] = \varepsilon_u b(\mathrm{molU/Vol.}) \tag{4-31}$$

$$A = \frac{\varepsilon_u b(\text{orig. mol U} - \text{mol T added})}{V_t + V_u} \tag{4-32}$$

$$A = \frac{\varepsilon_u b(V_u[\mathrm{U}]_o - V_t[\mathrm{T}]_b)}{V_t + V_u} \tag{4-33}$$

where $[U]_o$ is the original concentration of U before addition of any T ($[U]_o$ is the concentration that is usually determined in the analysis), $[T]_b$ is the concentration of T in the buret, V_t is the volume of titrant added, *not* the total volume, and V_u is the original sample volume (a constant). If one were to plot A *vs* V_t, the "normal" procedure for a titration curve, the resulting plot would not be linear. The reason for this is that the volume in the titration flask increases as the titration proceeds due to addition of titrant. However, if the measured absorbance is corrected for dilution by multiplying with the inverse of the dilution factor, $(V_t+V_u)/V_u$, then

$$\frac{A(V_t + V_u)}{V_u} = \varepsilon_u b[\mathrm{U}]_o - \frac{\varepsilon_u b[\mathrm{T}]_b V_t}{V_u} \tag{4-34}$$

The absorbance corrected for dilution is called simply the corrected absorbance, A_{corr}.

$$A_{corr} = \frac{A(V_t + V_u)}{V_u} \tag{4-35}$$

In most linear titration methods the response, both before and after the equivalence point, must be corrected for dilution of the solution as titrant is added. This correction is *always* done using an expression similar to Eqn. (4-35). This leads to the following expression.

$$A_{corr} = \varepsilon_u b[\mathrm{U}]_o - \varepsilon_u b[\mathrm{T}]_b V_t/V_u \tag{4-36}$$

Thus a plot of A_{corr} *vs* V_t will be *linear* with a slope of $-\varepsilon_u b[\mathrm{T}]_b/V_u$ and intercept of $\varepsilon_u b[\mathrm{U}]_o$.

After the equivalence point:
There is no longer any U present, thus there is no coloured material in solution and the absorbance will be zero.

$$A_{corr} = 0 \tag{4-37}$$

This is obviously another straight line but with zero slope and zero intercept.

The intersection of the line representing the response before the equivalence point (Eqn. (4-36)) and that after the equivalence point (Eqn. (4-37)) is obtained by setting Eqn. (4-36) equal to Eqn. (4-37).

$$\varepsilon_u b[U]_o - \varepsilon_u b[T]_b V_t/V_u = 0 \qquad (4\text{-}38)$$

or

$$[U]_o = [T]_b V_t/V_u \qquad (4\text{-}39)$$

This relationship applies only at the equivalence point, and thus one concludes that the two lines intersect at the equivalence point. The value of V_t in Eqn. (4-39) is that at the intersection, which is the value at the equivalence point, $(V_t)_{ep}$. A graph of the titration curve (the two straight lines) is shown in Figure 4-23.

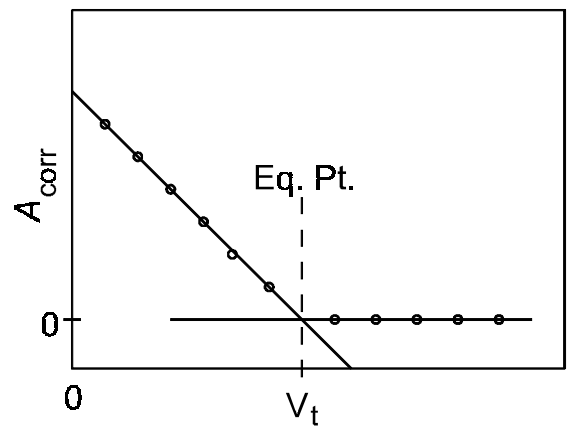

Figure 4-23

In this type of titration points are taken throughout the titration, but *not* at the equivalence point, so there is no need to approach this point carefully. Thus these titrations can be carried out quite rapidly. The equivalence point is determined by extrapolation of the two straight lines. This extrapolation is usually not as accurate as a good indicator colour change. However, this type of titration is free of indicator errors, which appear when the analyte concentration becomes low. Thus these titrations are most useful for the analysis of dilute solutions, and work quite well, since spectrophotometry is a sensitive technique.

Such titration curves will always be two straight lines, but their exact shape will depend on what substances are coloured, and how highly coloured each substance is. Two examples are shown in Figure 4-24. The equations for these curves can be derived in the same fashion as was done above.

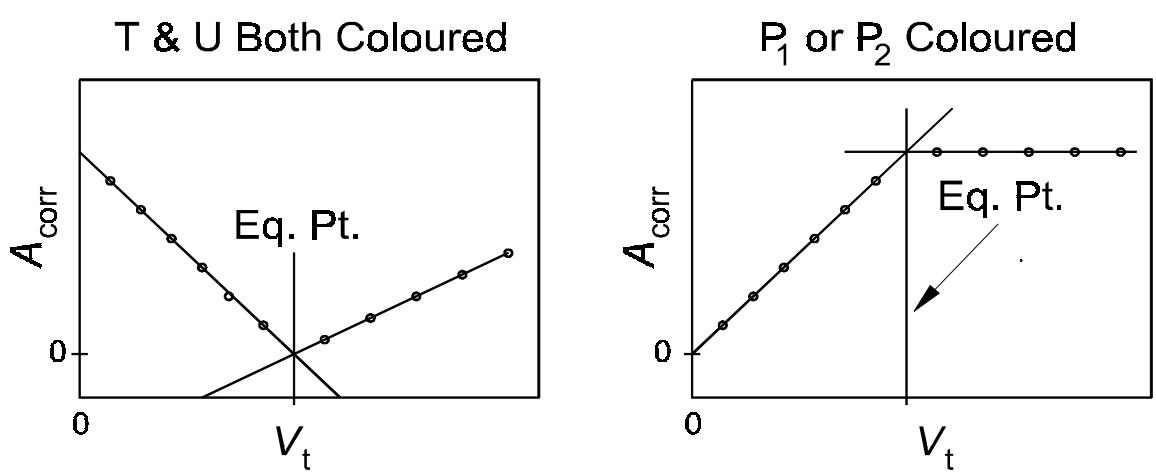

Figure 4-24

Consider the reaction:

$$2T + U \rightarrow 2P_1 + P_2$$

Derive equations for the two straight line segments of the linear spectrophotometric titration curve for the case where the only coloured material in solution is P_1. Prove that the equivalence point occurs at the intersection of these two lines. Assume that the cell path length is 1.00 cm.

Let ε_{P1} simply be represented as ε. For the segment before the equivalence point the only coloured species present is P_1:

$A = \varepsilon[P_1]$

$[P_1]$ = (mol of T added)/(total vol.) = $V_t[T]_b/(V_t+V_u)$

$A = \varepsilon V_t[T]_b/(V_t+V_u)$

Correct for dilution by multiplication of both sides by $(V_t+V_u)/V_u$

$A(V_t+V_u)/V_u = A_{corr} = \varepsilon[T]_b V_t/V_u$

A plot of A_{corr} *vs* V_t is linear with zero intercept and a slope of $\varepsilon[T]_b/V_u$. For the segment after the equivalence point the only coloured species present is still P_1:

$A = \varepsilon[P_1]$

$[P_1]$ = 2(mol U orig.)/total vol. = $2V_u[U]_o/(V_t+V_u)$

$A = 2\varepsilon V_u[U]_o/(V_t+V_u)$

After correction for dilution

$A(V_t+V_u)/V_u = A_{corr} = 2\varepsilon[U]_o$

A plot of A_{corr} *vs* V_t will be linear with zero slope and an intercept of $2\varepsilon[U]_o$. At the intersection of these two lines $(A_{corr})_{before} = (A_{corr})_{after}$ or

$\varepsilon[T]_b V_t/V_u = 2\varepsilon[U]_o$

$[T]_b(V_t)_{ep} = 2[U]_o V_u$ or mol T = 2(mol U)

which holds only at the equivalence point.

4.3 MOLECULAR EMISSION SPECTROSCOPY

As mentioned above, molecules can emit light, as well as absorb it, but to do so they must first be promoted to an excited state. There are various ways of doing this; one obvious way is to shine light upon the molecules. When the light is absorbed, the molecules are promoted to an excited state. Most molecules simply convert this absorbed energy to heat (kinetic energy) through collisions with other molecules in solution. (Processes in the solid or gaseous states will not be discussed.) However, some molecules will re-emit the energy as light. This process is described by the equation below.

$$h\nu + \mathrm{M} \rightarrow \mathrm{M}^* \rightarrow \mathrm{M} + h\nu'$$

If this process is fast (approximately 10^{-8} s), it is called *fluorescence*. This is the method used most often for molecular emission spectroscopy in solution and the only type that will be discussed. Almost always

$$\nu > \nu'$$

In other words, the emitted photon will almost always have less energy (it will have a longer wavelength) than the absorbed photon. This will turn out to be very convenient for the purposes of analysis. The reason for this phenomenon is shown in the stylized energy level diagram depicted in Figure 4-25.

Immediately after absorbing the exciting photon, the molecule will often be in an excited vibrational state (it *must* be in an excited electronic state). This vibrational energy is converted to kinetic energy very rapidly and the electronically excited molecule falls to its lowest vibrational state. This occurs before re-emission of a photon and is called vibrational relaxation. After the emission of the photon occurs, the molecule is returned to the ground electronic state, but may well be in an excited vibrational state. This extra vibrational energy is again degraded to heat through vibrational relaxation. The net result of this is that some of the energy of the exciting photon is converted to heat by vibrational relaxation, and the re-emitted photon will have a longer wavelength or lower energy than the exciting photon.

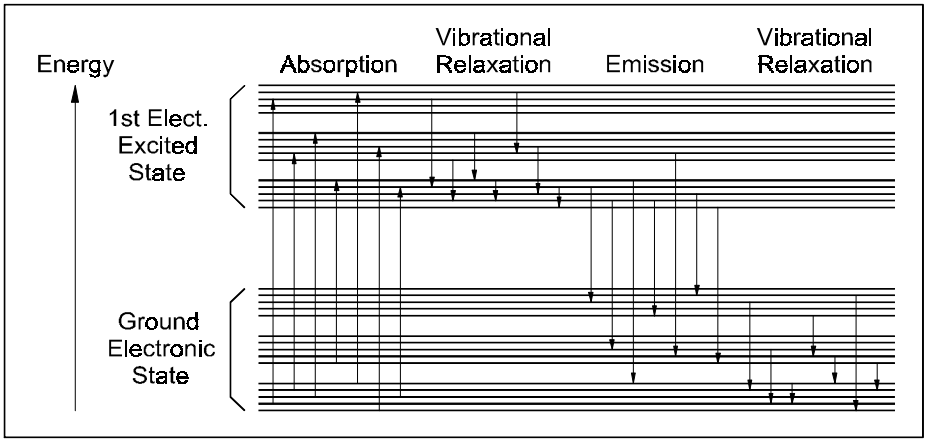

Figure 4-25

If both the absorption spectrum and the emission spectrum are plotted on the same graph, as shown in Figure 4-26, (the absorption spectrum is a plot of absorbance, A, *vs* λ, the emission spectrum a plot of emission intensity, I, *vs* λ), the effect is observed as a shift of the emission spectrum to longer wavelengths than the absorption spectrum. This difference allows the exciting light to be separated from the emitted light. This is absolutely necessary in order to use of this technique in trace chemical analysis.

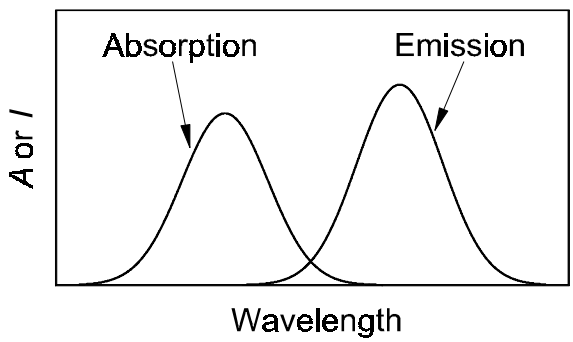

Figure 4-26

4.3.1 INSTRUMENTATION

As in absorption spectrophotometry, the instrumentation and the method of making the measurement are discussed first. A block diagram of the instrument is shown in Figure 4-27, followed by a brief description of the various components of the instrument. The light source provides polychromatic excitation radiation. The excitation monochromator selects the proper wavelength of light for the excitation process and this light passes through the sample cell (from left to right in

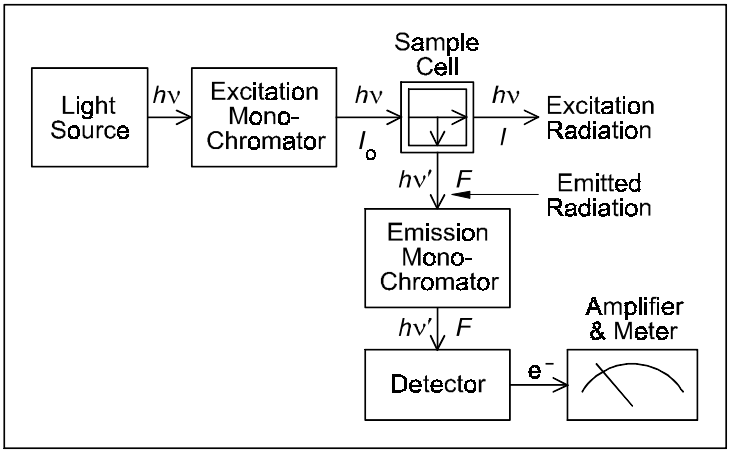

Figure 4-27

Figure 4-27), where some of it is absorbed, producing molecules in the excited state. The excited molecules fluoresce, emitting light in all directions. In most instruments the light emitted at right angles to the exciting radiation is collected and passes through the emission monochromator, which selects a portion of the emitted light to pass to the detector. The detector converts the light signal to an electrical signal which is amplified and presented to the analyst by the amplifier and meter.

Light source

In fluorescence one usually needs fairly energetic photons to excite the sample molecules, so tungsten filament lamps are seldom used. Almost all light sources are gas discharge tubes. These may contain H_2 or D_2, as in ordinary uv spectrophotometers, but more often contain either Hg or Xe. Xenon gas discharge lamps have the advantage that they emit both in the uv and the visible.

Monochromators

Unlike spectrophotometers, fluorometers contain two monochromators. The excitation monochromator selects a band of light which is strongly absorbed by the analyte. This light is highly sensitive for exciting the sample molecules. The emission monochromator selects a band of light centred on the emission band, and thus provides high emission sensitivity. These two bands must be completely separate, and the monochromators are set so that this is so, see Figure 4-28. As in Figure 4-26, Figure 4-28 presents both the absorption spectrum and the emission spectrum on the same graph. Only exciting radiation within the excitation bandwidth is allowed to enter the cell. Only fluorescence within the emission bandwidth is allowed to reach the detector. If these two bands overlapped, some of the exciting light, which is present in much higher intensity than the emitted light, might be scattered into the detector and give a false reading.

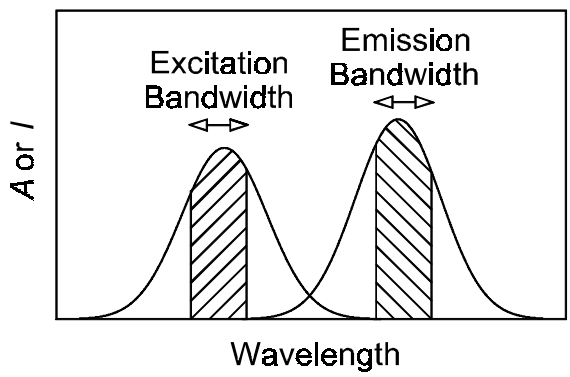

Figure 4-28

Often grating monochromators are found on instruments used in fundamental research on fluorescence and photochemistry. However, for simple analytical procedures filters are often used as monochromators. The reason for this is that filters pass much more light than grating monochromators and thus provide more sensitive and simpler instruments. A narrow bandwidth, as provided by a grating monochromator, is not nearly as important in fluorescence as in absorption spectrophotometry.

Sample cell

Square 1.00 cm cuvettes are usually used as sample containers. The difference between those used in fluorescence and absorption measurements is that silica is always used in fluorescence (because the exciting radiation is usually in the uv) and all four sides of the cuvette must be of high optical quality, because fluorescence is observed through the side at right angles to the side which passes the excitation radiation. Only two sides of an absorption cuvette need be of high optical quality. Observation of fluorescence at right angles to excitation helps to prevent interference of the excitation radiation with the emitted radiation.

Detector

A device called a photomultiplier tube is used as the detector in fluorescence. These operate on the same principle as vacuum photodiodes, except that they have internal electrodes that amplify the small electron signal from the photocathode (by a factor of 10^4 to 10^6). Thus they provide much larger currents for the same light input than photodiodes. This is needed since the light levels in fluorescence are very low.

Amplifier and readout

These are basically the same as in absorption spectrophotometry.

4.3.2 QUANTITATIVE RELATIONSHIPS

The next subject to be examined is the relationship between the light emitted by the sample and the concentration of analyte in the sample. The approach taken here will be a bit simpler than is taken in many texts; in other words more simplifying assumptions will be made. At low concentrations, where fluorescence is most useful, the results are the same, regardless of the approach.

In the first place, one would expect that fluorescence, F, (the intensity of secondary photons emitted) would be proportional to the intensity of primary photons absorbed. Mathematically

$$F = k(I_o - I) \tag{4-40}$$

It will be assumed that the sample molecules do not absorb any of the light which they emit. This is often a very reasonable assumption and when self-absorption does occur, the effect can often be nullified by proper setting of the emission monochromator. It will also be assumed that the entire sample cell is involved in the measurement, not just the centre portion. This will depend on the optics of the instrument. Both assumptions simplify the mathematics.

The proportionality constant, k, in Eqn. (4-40) is a function of the fluorescence efficiency, the number of photons emitted per photon absorbed (called the *quantum efficiency*), and the efficiency of light collection and detection. k is usually between 0.01 and 0.0001, indicating that the detector will see only one photon for between 100 and 10,000 photons absorbed. As will be pointed out below, fluorescence is almost always done at analyte concentrations far below those at which all exciting photons are absorbed. The conclusion one must reach is that usually the detected fluorescence is approximately four to six orders of magnitude or less in intensity than the exciting radiation.

If Beer's law is obeyed,

$$I = I_o \exp\{-2.303\varepsilon bc\} \tag{4-41}$$

$$F = k(I_o - I_o \exp\{-2.303\varepsilon bc\}) = kI_o(1 - \exp\{-2.303\varepsilon bc\}) \tag{4-42}$$

$$F = F_o(1 - \exp\{-2.303\varepsilon bc\}) \tag{4-43}$$

Eqn. (4-43) will be used later.

$$\frac{F_o - F}{F_o} = \exp\{-2.303\varepsilon bc\} \tag{4-44}$$

or

$$\ln\left(\frac{F_o - F}{F_o}\right) = -2.303\varepsilon bc \tag{4-45}$$

finally

$$c = \left(\frac{1}{2.303\varepsilon b}\right)\ln\left(\frac{F_o}{F_o - F}\right) \tag{4-46}$$

The net result of this treatment is to demonstrate that overall, even with certain simplifying assumptions, the relationship between F and c is fairly complex and non-linear. This is shown in Figure 4-29. Note that F_o is a constant and is the maximum fluorescence which can be obtained from a sample in the absence of self-absorption.

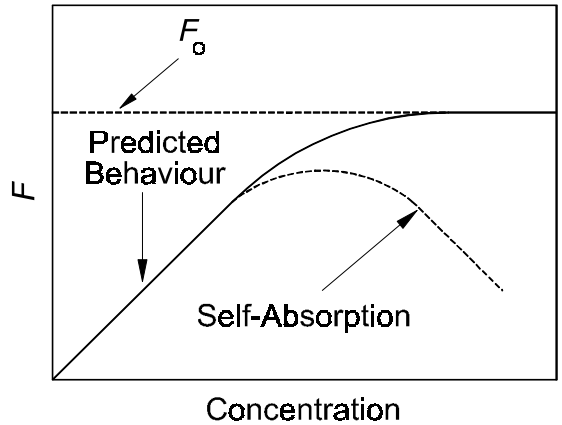

Figure 4-29

Is there a region in which F is linear in c? Yes, and this can be predicted mathematically. Return to Eqn. (4-43),

$$F = F_o(1 - \exp\{-2.303\varepsilon bc\}) \qquad (4\text{-}43)$$

and also note that exponentials can be approximated by power series. Thus

$$e^x = 1 + \frac{x}{1!} + \frac{x^2}{2!} + \frac{x^3}{3!} + \cdots \qquad (4\text{-}47)$$

In this case $x = -2.303\varepsilon bc$. If x is small enough, $e^x \approx 1 + x$.

$$F = F_o(1 - 1 + 2.303\varepsilon bc - (2.303\varepsilon bc)^2/2! + \ldots) \qquad (4\text{-}48)$$

And if $2.303\varepsilon bc$ is small enough,

$$F = F_o(1 - 1 + 2.303\varepsilon bc) = 2.303F_o\varepsilon bc = k'c \qquad (4\text{-}49)$$

The final conclusion is that fluorescence intensity is linear in concentration at low concentrations. Can this be explained in a less mathematical fashion? Imagine that you are looking at the sample cell from the same direction as the detector, that is at right angles to the exciting light (see Figure 4-30). If the concentration of analyte is very low, $c = c_1$, the absorption of light is also low and the exciting light intensity is the same from left to right across the cell. Thus all analyte molecules are subjected to the same excitation intensity and the emission intensity is uniform across the cell. Doubling the analyte concentration will double the fluorescence. However, as the analyte concentration gets higher, $c = c_2$ or c_3, more of the exciting radiation gets absorbed as it crosses the cell. Molecules to the right are subjected to less excitation intensity, and thus emit less than molecules to the left of the cell. This gives rise to a "negative deviation" from the linear equation. As the concentration of analyte increases still more, this effect gets more serious, until some concentration is reached, $c = c_4$, at which all exciting photons are absorbed in the cell. At this point molecules on the right wall of the cell see no exciting radiation and emit no fluorescence. From this point on increasing concentration will provide no more fluorescence since all exciting radiation is absorbed.

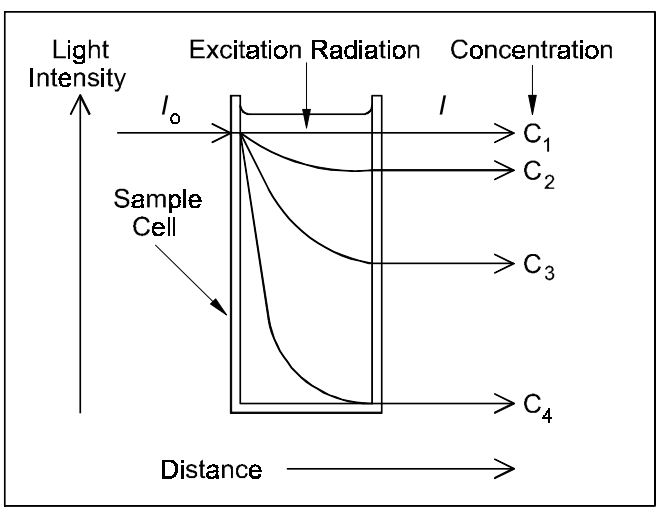

Figure 4-30

This line of reasoning leads to the conclusion that the region over which fluorescence is linear in concentration is that in which concentrations are low enough that absorption spectrophotometry may give poor results. In fact, fluorescence is a much more sensitive technique than absorption spectrophotometry. The question now arises "If fluorescence radiation is four to six orders of magnitude less intense than the exciting radiation (the light that would be used in absorption measurements), how can fluorescence be more sensitive?" The answer will be discussed below.

4.3.3 PRACTICAL FLUORESCENCE

The actual measurements are made in much the same way as measurements are made in absorption spectrophotometry (except that there is no absolute method). Thus one prepares a blank, one or a set of standards, and the sample. The instrument reading can be set to zero with no sample in the exciting light beam, or it can be set to zero with the blank in the light beam. In the first case the blank fluorescence must be measured and subtracted from the fluorescence of all the standards and the unknown (in some cases the sample and the standards may have different blanks). In the second case the instrument subtracts the blank fluorescence from the sample. A calibration curve is prepared from the standards and used to convert the measured fluorescence of the sample to concentration. The method of standard addition can be used, *if you are sure that fluorescence is linear in concentration*.

The final points to be discussed are concentration range, accuracy, and usefulness. From what has been said it might seem that fluorescence would be almost impossible to use. It is linear only when the concentrations are so low that very little of the exciting radiation is absorbed. In other words it is linear when $I \approx I_o$. Secondly, the fluorescence intensity is very low, requiring a very sensitive instrument. Why not use absorption spectrophotometry instead, since all molecules that fluoresce must also absorb light? (In fact, absorption spectrophotometry is used more often than fluorescence.) If one wishes to make measurements at very low concentrations with absorption spectrophotometry, one must measure both I and I_o very accurately and there must be no noise in the instrument, because the absorbance depends on the difference between the logarithms of these two intensities ($A = \log I_o - \log I$). In fluorescence, even though F is usually very low, the blank is, or can be set to, zero. It is much easier to distinguish between zero and a small intensity and to make accurate measurements of that small intensity than to distinguish small differences between two large intensities and make an accurate measurement of that difference.

Fluorescence is a very sensitive analytical technique. It is perhaps two orders of magnitude more sensitive than absorption spectrophotometry and is used in the range 10^{-8} to 10^{-5} M. It is more specific than absorption spectrophotometry, since fewer molecules fluoresce than absorb. It is used most often in biochemical analyses for molecules such as drugs and neurotransmitters. Sometimes derivatives that fluoresce can be made from non-fluorescent materials. A good example is the use of a material called dansyl chloride which, when reacted with amino acids, forms products which fluoresce.

Fluorescence is also very "fiddly". Since it is such a sensitive method, samples are easily contaminated by very small amounts of impurities which fluoresce. Also many real world samples contain more than one fluorescing substance, thus there are often background or matrix problems. Great care must be taken to prevent contamination. The usual accuracy to be expected is between about 2 and 10%.

In a determination of quinine by fluorescence, solutions of 5.0×10^{-7} M and 1.0×10^{-6} M quinine sulfate are prepared. A blank is used to set the fluorometer to zero. The two quinine standards are then read as 39.6 and 79.5 (in arbitrary emission units) respectively. Is fluorescence linear in concentration? A sample of tonic water gives a fluorescence reading of 67.5. What is the concentration of quinine in the tonic water?

Since the second standard concentration is double the first, and since the second intensity is almost also exactly double the first, one would say that fluorescence is linear in concentration. A more elegant method of proving the same thing is as follows. If F is linear in c

$F = k'c$ or $k' = F/c$

$k'_1 = 39.6/5.0\text{x}10^{-7} = 7.92\text{x}10^{7}$

$k'_2 = 79.5/1.0\text{x}10^{-6} = 7.95\text{x}10^{7}$

Since k'_1 and k'_2 are within 3 parts in 800 of each other, linearity can be assumed. $k_{avg} = 7.935\text{x}10^{7}$

$c_{unk} = F/k_{avg} = 67.5/7.935\text{x}10^{7} = 8.5\text{x}10^{-7}$

A plant extract is to be analyzed for a certain alkaloid as follows: 10.00 mL of the extract are diluted to 20.00 mL with distilled water and produce a reading of 46.3 (arbitrary emission units) on the fluorometer. 1.00 mL of a $1.00\text{x}10^{-6}$ M solution of the alkaloid is added to 10.00 mL of extract and diluted to 20.00 mL with distilled water. This solution reads 51.4. Finally 10.00 mL of the extract are mixed with 5 mL of a reagent with destroys the fluorescence of the alkaloid and this is diluted to 20.00 mL with distilled water. This solution reads 20.8. What is the concentration of the alkaloid in the extract (assume that *F* is linear in *c*)?

The blank equals 20.8 and this must be subtracted from the other fluorescence readings.

$F = 46.3 - 20.8 = 25.5 = k'c = k'c_{unk}(10.00\text{ mL})/(20.00\text{ mL})$

$F = 51.4 - 20.8 = 30.6 = k'(c_{unk}(10.00)/(20.00) + (1.00\text{x}10^{-6}\text{ M})(1.00\text{ mL})/(20.00\text{ mL}))$

$30.6/25.5 = 1 + 1.00\text{x}10^{-6}\text{ M}/10.00c_{unk}$

$c_{unk} = 1.00\text{x}10^{-7}\text{ M}/(30.6/25.5 - 1.00) = 5.00\text{x}10^{-7}\text{ M}$

4.4 ATOMIC SPECTROSCOPY

Atoms absorb and emit light in the same fashion as molecules (in the visible and uv), except that this process is uncomplicated by rotational and vibrational transitions, making it appear much simpler. On absorbing a photon, an atom is promoted to a more energetic electronic state and on emitting a photon an atom falls to a less energetic electronic state. Often the ground state is one of the two states involved in these transitions, but not always. These phenomena should therefore be able to be used to perform quantitative analysis for atoms in the same fashion that has been used for molecules. However, in terms of analysis, two major differences exist between molecular and atomic spectroscopy.

1) Atomic absorption and emission lines are very narrow compared to the corresponding molecular bands in solution, as explained in Section 4.1.3. This is both a “problem” and a “feature”. It is a feature in that with such narrow lines, very few lines overlap. If one has many different coloured molecular species present in solution, their absorption bands would certainly overlap and analysis without a prior separation step would not be possible. One can analyze many metals in the presence of each other without any separation step because their lines do not overlap.

It is a problem in that it is impossible to make a monochromator good enough to provide light of sufficient purity for Beer’s law to be obeyed, since the monochromator bandwidth must be narrow compared to the absorption bandwidth.

2) Molecules exist readily in solution, *atoms do not*. Thus some method must be devised to convert molecular or ionic species into atoms in order to do the spectroscopy. This will undoubtedly cause drastic changes in the sample.

4.4.1 ATOMIC ABSORPTION SPECTROSCOPY

Just as in molecular spectroscopy, the topic of atomic absorption (AA) is taken up first. One might guess that the instrument for atomic absorption would be the same as that for molecular absorption - source, monochromator, sample, detector, amplifier, readout. In some respects this is so, but the instrument must somehow be constructed in a manner to take into account the two differences mentioned above.

Atomic bandwidth

The problem of bandwidth is examined first. How narrow is an atomic line? Atomic lines have an inherent bandwidth set by the uncertainty principle at about 10^{-4} nm (compared to molecular bandwidths in solution of about 100 nm). However, the observed bandwidth is much larger than this for two reasons:

1) Doppler broadening. This is a broadening of the absorption line caused by motion of the atoms with regard to the light source (or the direction of the light ray) in AA or with regard to the detector in atomic emission. Doppler broadening is a general phenomenon associated with wave motion. The classic example is the change in pitch of a grade crossing signal as heard by a train passenger. As the train approaches the signal source, the pitch is higher than would be heard by a stationary observer. As the train moves away from the signal source, the signal pitch is lower than would be heard by a stationary observer. In a similar fashion an atom moving toward a light source (moving in a direction opposite to the direction of the light ray) will experience a frequency higher than an atom at rest. Thus that atom will absorb at a frequency slightly lower (as measured by a stationary observer) than an atom at rest (remember that the atomic energy levels are not affected by translational motion). Likewise an atom moving away from a light source (moving in the same direction as the light ray) will experience a frequency lower than an atom at rest. That atom will absorb at a frequency slightly higher than it would at rest. This behaviour is depicted in Figure 4-31, where ν is the actual frequency of the light ray and ν' is the frequency experienced by the moving atom. Atoms moving at right angles to the light ray will absorb the "correct" frequency. Thus the width of the band of frequencies absorbed by moving atoms is broader than if the atoms were stationary. This type of broadening is temperature dependent, since velocity is a function of temperature.

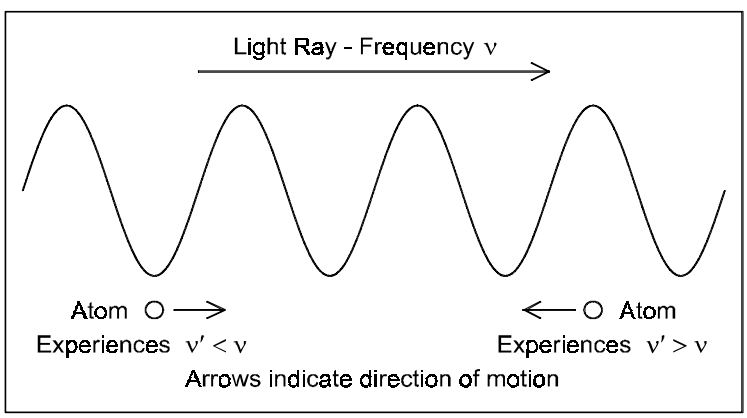

Figure 4-31

2) Pressure broadening. The energy states or levels in atoms are not distorted by translational motion, but they are distorted by collisions. This distortion causes these states to be somewhat "fuzzy", not having the expected exact energy values. This in turn affects the energy differences between states and the frequencies of light interacting with these states. This type of broadening is almost directly

proportional to pressure. These two mechanisms combine to produce atomic linewidths in the range of 10^{-3} to 10^{-2} nm.

How does one obtain light with such a narrow bandwidth in order to make absorption measurements? *Not with a monochromator!* One must use a special light source that produces a line of exactly the correct wavelength and a very narrow bandwidth. To produce a line of the correct wavelength, the light source must operate with some form of gas discharge and must contain the same element (atom) that is in the sample (the analyte). One such light source is the hollow cathode lamp (HCL).

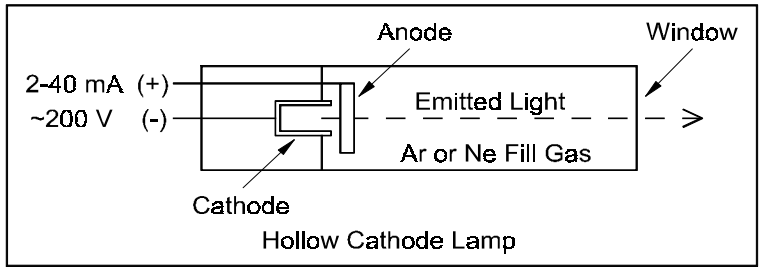

Figure 4-32

Such lamps (see Figure 4-32) usually contain either Ar or Ne gas at a low pressure (0.001 to 0.005 atm). The cathode (the negative electrode) is a hollow cylinder made from or containing the atom of interest, *e.g.* Cu. A gas discharge in the HCL creates Ne^+ and e^-, which move toward the cathode and anode respectively. When the neon ions collide with the cathode, they sputter some Cu into the space within the hollow cylinder. These copper atoms can then be excited by collision with Ne^+ or e^- and emit light characteristic of copper.

$$Cu + Ne^+ \text{ (high energy)} \rightarrow Cu^* + Ne^+ \text{ (lower energy)}$$

$$Cu^* \rightarrow Cu + h\nu$$

Some collisions are energetic enough to form excited copper ions which can also emit light.

$$Cu + Ne^+ \rightarrow Cu^{+*} + Ne^+ + e^-$$

$$Cu^{+*} \rightarrow Cu^+ + h\nu'$$

In addition to excited copper atoms and ions there will also be excited neon atoms and ions, and perhaps some other atoms and ions depending on the construction of the HCL. Thus not only will one obtain the radiation characteristic of copper, the element of interest in the example, but also that radiation characteristic of copper ions and of neon atoms and ions. This latter radiation is of no use in the atomic absorption spectroscopy of copper atoms. How the problem of this "extra" radiation is solved will be discussed later. Those lines that are of use for the determination of copper are narrow enough that Beer's law is obeyed reasonably well.

Sample atomization

The second difficulty, that of converting the sample to atoms, is now examined. The solution to the problem is relatively straight forward - one heats the sample sufficiently to destroy the sample matrix (molecules or crystal lattice) and produce atoms. There are two common methods of doing this in AA, (1) use a small oven, usually made of graphite, and (2) introduce the sample into a flame.

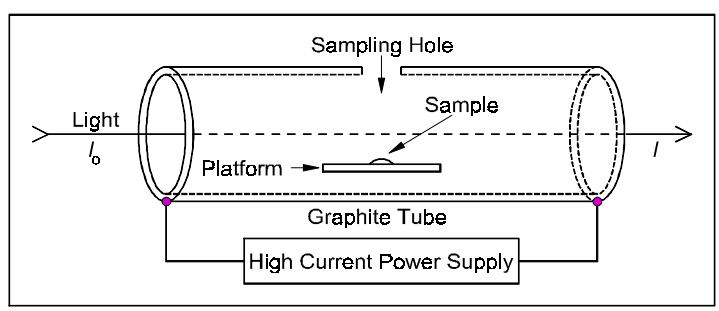

Figure 4-33

1) Graphite furnace (electrothermal) atomization. There are several different models of these furnaces available commercially. In the example shown in Figure 4-33, a small sample, usually < 100 μL, is placed on a platform inside a graphite tube. While an inert gas is passed over and through the tube to protect it from oxidation during heating, an electric current is passed through the tube causing the temperature to rise. As the furnace is heated the solvent evaporates, then any organic matrix decomposes ("smoke" may be produced), then the inorganic matrix decomposes and the sample is atomized. These atoms then absorb the light passing through the tube from the hollow cathode lamp while the tube acts as the sample cell. The entire experiment occurs fairly rapidly and the absorbed light appears as a pulse or peak, not a continuous signal. The peak height of this pulse, when measured as absorbance, is usually proportional to sample concentration. This process is depicted in Figure 4-34. The upper plot indicates the variation of temperature with time in the furnace. The lower plot indicates the variation of measured absorbance with time. The "smoke" caused by matrix decomposition appears as an absorption band earlier in time than the actual absorption band. In fact the "smoke" may either absorb the light or scatter it, the result is the same. This peak is ignored in the analysis.

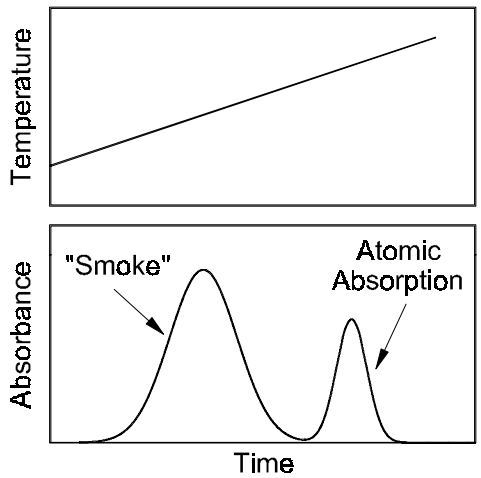

Figure 4-34

This type of atomization is very sensitive and capable of analyzing small samples, 20 μL or less. It is also rather exacting and demands great care and precision. Best results are achieved with automated sampling.

2) Flame atomization. In this type of atomization, sample is introduced into a nebulizer by passing a high flow of oxidant gas (usually air) past the tip of a small capillary connected to the sample (see Figure 4-35). At the capillary tip the sample is broken into a fine mist. The smallest particles in this mist are carried into the flame by the gas flow, the largest fall out of the gas stream and eventually exit through the drain. In most such burners only about 10% of the sample gets into the flame. Fuel gas, usually acetylene (ethyne), is also introduced into the nebulization chamber and mixes with the sample mist and the oxidant. This produces a premixed flame which burns quietly and evenly (the flame on a bunsen burner is premixed).

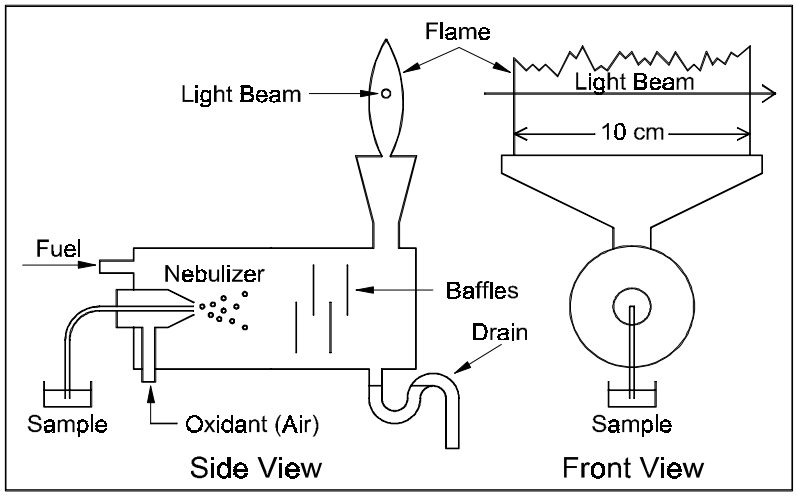

Figure 4-35

In the flame the solvent in the mist particles evaporates first. Then the solid remaining is atomized (providing that its crystalline form is not too refractory). The atoms thus formed absorb the light passing through the flame from the hollow cathode lamp. The aspiration of sample into the flame is continuous and thus the signal produced by the absorption of light is also continuous, as contrasted with that produced in the graphite furnace. In effect the flame becomes the sample cell.

Flame atomization is not as sensitive as electrothermal atomization and requires much larger amounts of sample (1 - 10 mL), but is simpler and more easily done by hand.

Instrumentation

With this introduction to hollow cathode lamps and atomization it is now possible to consider the entire instrument used for atomic absorption and shown in Figure 4-36 in block form. The detector (usually a photomultiplier tube), amplifier, and meter (readout) are basically the same as those discussed previously. The hollow cathode lamp and the atomizer have just been described. A flame atomizer is depicted in Figure 4-36. If a graphite furnace were to be used in place of the flame, it would go in the same location. However, two features appear that have not been observed or discussed previously. The instrument shown in Figure 4-36 has a device called a chopper placed in the light beam and the monochromator follows the sample rather than preceding it. With a hollow cathode lamp as source, it might at first seem that the monochromator is not needed.

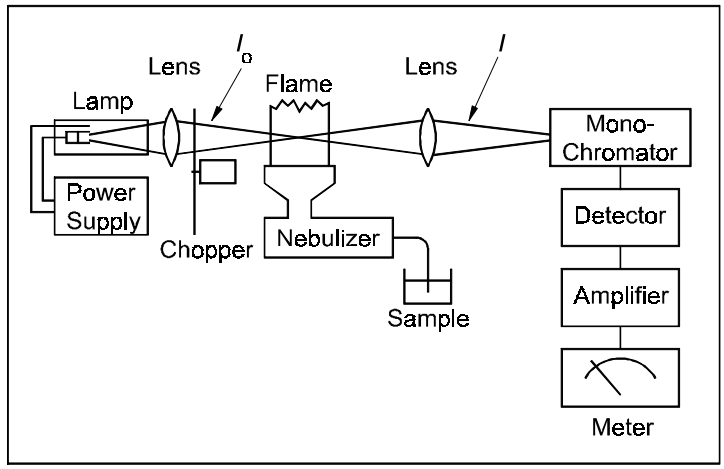

Figure 4-36

Light beam modulation

The need for a chopper (light beam modulation) is discussed first. As an introduction to this topic note that most spectrophotometers and all fluorometers place the sample cells in light-tight compartments. Under this circumstance room light can have no effect on the measurement. However, it is impossible to place flames and hot graphite tubes in light-tight compartments. Even if this could be done, it would not solve the problem since the flames and graphite furnaces are sources of light themselves. Thus room light and light given off by the flame or furnace will interfere with the measurement and some method must be found to differentiate such light from the signal light (light from the HCL). This is done by a technique called modulation.

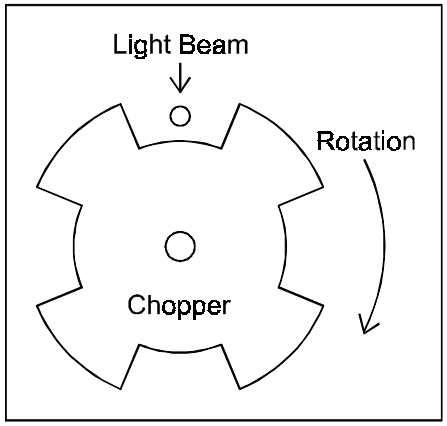

Figure 4-37

The light from flames and furnaces and much of that found in the laboratory is steady in nature, its intensity does not vary with time. If the light from the HCL is modulated (the light intensity is made to vary with time in a periodic fashion), then light from the HCL and from the interfering sources can be distinguished. The chopper is one type of device that can introduce the modulation (see Figure 4-37). It is similar to a fan whose blades are placed in the HCL light beam. When the chopper rotates, the light is alternately stopped by the blades and passed between the blades. Before the chopper the light intensity is steady, after the chopper the intensity alternates between on (high) and off (low). This alternation is called modulation. The frequency of the modulation depends on the rotation rate of the

chopper. The amplifier is special in that it can be exactly "tuned" to the frequency of the light produced by the chopper, much like a radio is tuned to a specific broadcasting station. Thus the amplifier passes only the signal from the HCL and none of the background signal, and in this way gets around the problem of light from sources other than the HCL. This is depicted graphically in Figure 4-38.

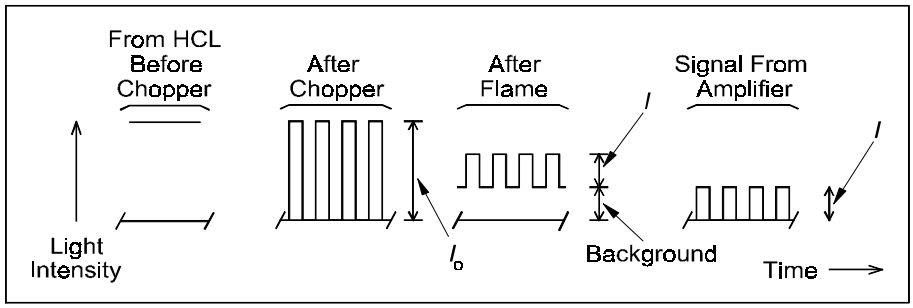

Figure 4-38

Almost all modern research instruments no longer use a chopper. Instead the power supply to the hollow cathode lamp is pulsed. This also produces pulsed or modulated light from the HCL, and the net result is exactly the same as if a chopper were used.

Monochromator

It has been stated that no monochromator can provide light with a narrow enough bandwidth (less than 10^{-3} nm) to be used in atomic absorption. A hollow cathode lamp is needed to provide light of narrow enough bandwidth. However, one difficulty with HCLs, as already mentioned, is that they not only produce light from the element of interest (*e.g.* Cu), but also from ions of this element (Cu^{+} or Cu^{2+}) and from atoms and ions of the fill gas (*e.g.* Ne, Ne^{+}, *etc.*) and any other element that might be present in the cathode. None of this latter radiation can be absorbed by the Cu atoms in the sample, and, if it gets through to the detector, it will cause the sensitivity of the measurement to drop dramatically and Beer's law to be invalid.

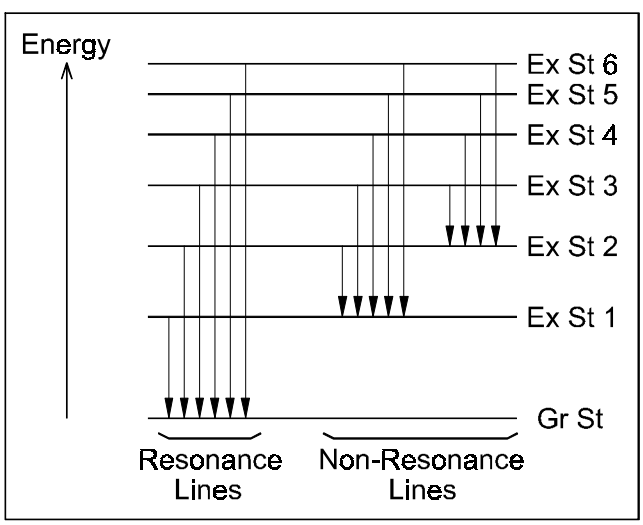

Figure 4-39

In addition not all of the light from the atoms of the element of interest can be used either. To explain why this is so, consider the simulated energy level diagram depicted in Figure 4-39. The arrows on this diagram represent the possible energies of the light rays emitted by the HCL from the element of interest. Those lines which terminate in the ground state are called resonance lines. Only the resonance lines and none of the other atomic lines can be absorbed by atoms in the flame or furnace since atoms in the flame or furnace are normally in the ground state before absorbing the light (remember that absorption has the arrows going in the direction opposite that for emission). The final conclusion is that very few of the lines produced by the HCL are useful in atomic absorption spectroscopy.

Some way must be found to select only the one line most useful for the measurement. This is the job of the monochromator in atomic absorption. *The monochromator does not make the line emitted by*

the HCL more monochromatic, it just selects the desired line. This is illustrated by Figure 4-40. When placed after the flame or furnace, the monochromator also helps to remove background light from the sample source and the room.

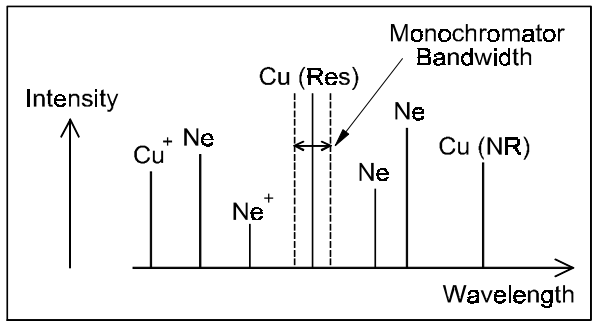

Figure 4-40

One question still needs to be answered - why is the emission bandwidth of the HCL narrower than the absorption bandwidth of the sample atoms in the flame or furnace (it must be if Beer's law is to be valid)? Remember that the atoms absorbing the light are at room pressure and are quite hot (in a flame or furnace). The pressure in the HCL is very low, and thus pressure broadening is not as pronounced as at room pressure. Also the translational temperature of atoms in the HCL is lower than the temperature in the flame or furnace, lessening the effect of doppler broadening.

Methods, uses, sensitivity, *etc.*

Absorption measurements in flames and furnaces are made in the same way as those in solution. Beer's law (Eqn. (4-10)) is the basic expression used to relate concentration and instrument response. Calibration curves are normally employed although standard addition can be used when Beer's law is obeyed. Unfortunately Beer's law is not as well obeyed in AA as in solution spectrophotometry. There are two reasons for this: (1) the bandwidth of the light from the HCL is not considerably narrower than that of the atoms in the atom source and (2) the atom source (flame or furnace) is not a very ideal sample cell. In solution work the boundaries of the cell are very well defined and the sample is uniform throughout the cell, Neither of these circumstances exist in flames or furnaces, and this causes deviations from Beer's law.

It is obvious that the sample must be destroyed in order to obtain the atoms for analysis. Therefore AA is useful for what is called *elemental analysis*, that is the analysis of the elemental content of the sample. Thus, in the case of copper, AA can determine the total amount of copper in a sample, but not how that copper is incorporated in the sample. It cannot determine one form of copper in the presence of two or more forms. Can AA determine all elements with equal ease? To answer this question one must first examine the spacing of energy levels in the various elements. In general the energy level differences in atoms get larger as one proceeds from the left of the periodic table (the alkali metals) to the right (the halogens) - from metals to non-metals. The alkali metals absorb in the visible region of the spectrum, the halogens in the vacuum ultraviolet. Most metals absorb in the ultraviolet. AA cannot be used in the vacuum ultraviolet (wavelengths below 200 nm) because these wavelengths are absorbed by the atmosphere. Thus AA is not used for the analysis of non-metals.

In terms of the analysis of metals, the sensitivity of absorption (the molar absorptivity) varies from element to element. More important, the ease with which materials can be broken into atoms varies from element to element. The refractory metals form materials, often oxides, that are very hard to break into atoms. The net result of this is that the materials containing these elements leave the flame or the furnace before being broken into atoms, and thus atomic absorption never occurs. Thus elements like zirconium and tantalum show low sensitivity whereas elements like copper and cadmium can be determined with high sensitivity.

Since the metals make up about 90% of the elements, AA can be used to determine about 90% of the entries in the periodic table. Like molecular absorption, AA is a trace technique, generally operating in the range of 10^{-5} to 10^{-8} M. Flame AA covers the higher end of this range and graphite furnace AA the

lower end. Electrothermal AA is one of the most sensitive techniques available for the determination of many metals. The usual expected accuracy of this technique, when properly applied, is between about 2 and 10%.

As with all analytical techniques, certain problems or characteristics must be taken into account when using atomic absorption. As pointed out above, AA is a destructive technique. The sample cannot be recovered in any form. This also means that no speciation information is available from this technique. Speciation information involves knowledge of the form in which the element exists in the sample. For example, in a sample containing chromium, is the chromium present in the +2, +3 or +6 oxidation state; is the chromium found as an oxide, a sulfide, a sulfate, *etc*.; is the chromium complexed with an organic ligand, is it a simple ion, or adsorbed on some other material? These questions cannot be answered by AA. All that AA can reveal is the total chromium present in the sample.

Atomic absorption is affected by certain matrix effects. Many elements show different absorbances depending on the other substances present in the sample. For example, Ca shows different sensitivity in Cl^-, SO_4^{2-}, and PO_4^{3-} solutions. The reason for this is that chlorides, sulfates, and phosphates atomize to different extents, and thus provide different numbers of atoms in the atom source.

This type of matrix effect can often be circumvented by adding a material to the sample which will provide the same atomization efficiency regardless of the other materials in the sample. Large organic ligands such as EDTA (ethylenediamine tetraacetic acid), which form strong complexes with metal ions, are often used in this regard. The other approach is to use standard addition, since in standard addition the same matrix is involved in both the sample and the standard, and the sensitivity does not change drastically between sample and standard.

Metals that are easily ionized also sometimes present problems. The alkali metals are the best examples of such elements. Ionization proceeds as shown below.

$$M \rightarrow M^+ + e^- \qquad K_e = [M^+][e^-]/[M]$$

As the temperature rises, the reaction proceeds more to the right and the value of the equilibrium constant increases. This reaction acts just like the ionization of a weak acid in solution - the percent ionization increases as the total concentration of M decreases. This is in reality a chemical deviation from Beer's law, which would appear positive if the measured absorbance were plotted *vs* total metal concentration in solution. Since the standards and samples may not contain the same substances, the concentration of electrons in the atom source for the sample and standards may not be the same, leading to different amounts of ionization in the sample and standards. This problem is circumvented by adding a large concentration of an easily ionized element like potassium to all solutions. In this case the electron concentration, which is established by the large and constant concentration of potassium, is constant. This in turn forces the ratio of $[M^+]/[M]$ to be constant, avoiding both the matrix and deviations from Beer's law problems. The addition of potassium buffers the electron concentration and acts in a fashion similar to a pH buffer in solution. The assumption made in this example is that potassium is not one of the elements to be analyzed. If this is not the case, other metals can be used in its place.

Since the method of solving problems in AA is the same as in visible - uv solution spectrophotometry, no examples will be presented.

4.4.2 ATOMIC EMISSION SPECTROSCOPY

As was the case for molecules, if atoms can be promoted to an excited state, by falling to a lower energy state they will radiate light which can then be used to identify the emitting element and to perform quantitative analysis. The difficulty is getting the atoms into an excited state. Thermal energy (heat) is the method normally used to excite the atoms since the sample has to already have been heated

to destroy the matrix and produce the atoms. There are two sources of thermal energy normally used in atomic emission, flames and plasmas.

Emission from flames

As was mentioned in discussing AA, most atoms in flames are in the ground state. However, flames produce enough energy to excite some metal atoms, especially those on the left of the periodic table, *e.g.* groups 1 and 2 (Ia and IIa). Even the atoms from these elements are mainly in the ground state, but enough are excited so that sufficient light is emitted by the flame to detect these metals at very low concentrations. This is the oldest of atomic spectroscopic techniques, going as far back as Bunsen in the 1800s.

In order to obtain maximum emission, one would use the hottest flame available. Table 4-4 lists some of the more useful fuel-oxidant mixtures and their flame temperatures.

Table 4-4. Flame Temperature (°C)

Fuel	Oxidant		
	Air	Oxygen	N_2O
C_2H_2	2400	3140	2800
H_2	2045	2660	2690

Obviously it would seem better to use either oxygen or nitrous oxide as oxidant because of the higher temperatures. However these oxidants, especially oxygen, cannot be used in a premixed burner (such as is used in AA) because the flame burns so rapidly that it would flash back in such a burner. (Nitrous oxide can be used in special premixed burners if precautions are taken.) This is unfortunate since a premixed flame burns more steadily and quietly and with less background radiation. In general only the alkali metals are determined in premixed flames with air as oxidant.

A "total consumption" burner is used when the flame components have a high burning rate. Such a burner is diagrammed in Figure 4-41. As with the nebulizers used in AA, the sample is nebulized by passing rapidly moving gases past the tip of a capillary dipping into the sample. The mist produced is introduced directly into the flame. The fuel and oxidant gases are also mixed directly in the flame (no premixing). The term "total consumption" is a bit misleading. It would appear that all the sample enters the flame, as opposed to only about 10% in the systems used in AA. However, there is strong reason to believe that most of the sample moves through such flames too rapidly to be atomized and thus their performance is no better than the nebulizer-burner systems used in AA. These burners produce hotter flames than premixed burners and can be used to excite more elements, but are seldom used today since plasma emission, described below, is an even better excitation medium.

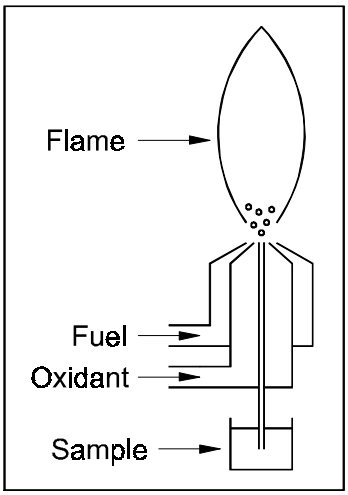

Figure 4-41

Emission from an ICP

"ICP" stands for inductively coupled plasma. A plasma is a very hot mixture of molecules, atoms, ions, and electrons in the gas phase. In the inductively coupled plasma intense microwave radiation (basically low energy heat radiation) heats a gas mixture to temperatures near 10,000 K. This heating is

similar to that in a microwave oven but the power levels and temperatures are much higher. Argon is used as the gas which forms the plasma. Liquid sample is introduced into the argon gas stream in the same way as in AA (in a nebulizer system similar to that shown in Figure 4-35) and is atomized in the plasma in a device called a plasma torch, shown in Figure 4-42. The high temperature of the plasma has two obvious advantages: (1) it can excite more elements than a flame and it excites each element to a greater degree, thus giving higher sensitivity, and (2) it almost completely destroys the matrix, causing many of the matrix effects observed in flames to disappear. However, many more spectral lines are observed from a plasma emission source and this can cause some spectral overlap and interference between elements. The ICP uses a large quantity of argon, which is expensive.

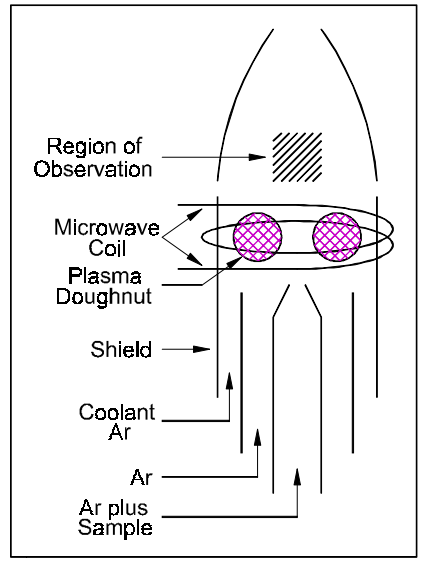

Figure 4-42

Instrumentation

The instrument used in this technique is very simple, especially if a flame is used as the emission source. The light from the emission source is focussed on the monochromator which in turn selects the line of interest and rejects all the other lines from the flame or plasma. The detector, amplifier and readout are similar to those used in the other spectroscopic techniques which have been discussed. In the more sophisticated instruments using an ICP, multielement analysis can be performed simultaneously. This is done using a monochromator with several exit slits. These are placed at different positions along the dispersed spectrum and select lines from different elements. For each exit slit and line, there is a detector, amplifier, and readout. Up to about 20 elements can be determined at one time for each sample. This type of analysis is of interest to geologists, mining companies, steel mills, *etc*.

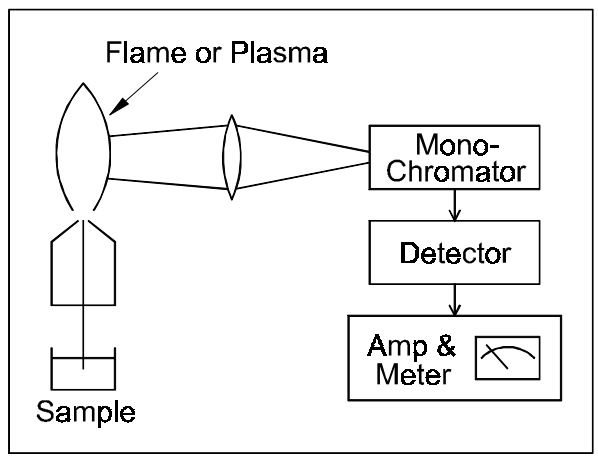

Figure 4-43

Methods, uses, *etc.*

In this type of emission technique (as compared to fluorescence), intensity is usually linear in concentration over many orders of magnitude.

$$I = kc \tag{4-50}$$

As in all spectroscopic techniques, blanks are used to determine the background signal level, and this level is subtracted from the signal provided by the sample. Usually several standards are employed to prepare a calibration curve (a plot of I *vs* c) and this curve is used to translate the emission intensity of the sample into concentration in the normal manner. Standard addition may also be used in atomic emission analysis.

The characteristics of atomic emission are very much like those of AA. It is used for the elemental analysis of metals in samples. It is a destructive method and provides, by itself, no information on speciation. It is a trace technique operating down to 10^{-7} M at accuracies of between 2 and 10%. Thus it might seem to be competitive with AA, and to a large extent it is.

The particular sample often determines which spectroscopic technique is best used. Flame emission instruments are very simple and reliable and are used most often to analyze the alkali metals. Flame AA instruments are also simple and relatively inexpensive and analyze a much wider range of elements than flame emission instruments. These are used when a specific element is to be analyzed and the highest sensitivity is not required. Recall that AA instruments usually only analyze one element at a time. For multielement analysis, for higher sensitivity, and for the analysis of refractory elements, the ICP-emission technique is best. Finally for the highest sensitivity (for non-refractory elements), and if sample sizes must be kept small, electrothermal AA is the technique of choice.

Lithium is often administered as a drug to reduce the symptoms of manic depression. A flame photometer is adjusted to zero (arbitrary intensity units) while aspirating a 0.0001 M KCl (a radiation buffer) solution which contains no lithium. A 1.00 ppm Li^+ solution, also containing 0.0001 M KCl, gives a reading of 86.7 units. 10.00 mL of a urine sample are mixed with 10.00 mL of 0.0002 M KCl and this solution produces a reading of 36.4 units. What is the lithium concentration in the urine sample?

$I = kc$

k = 86.7 aiu/1.00 ppm = 86.7 aiu/ppm

$c_{buffer} = I/k$ = 36.4 aiu/86.7 aiu/ppm = 0.420 ppm

c_{urine} = 0.420(20.00 mL)/(10.00 mL) = 0.840 ppm

4.5 PROBLEMS

Section 4.1: Introductory Problems

4-1) Calcium atoms absorb and emit light at a wavelength of 422.7 nm (among other wavelengths). What is the frequency and energy (per mole of photons) of this light? Ans: 7.093×10^{14} s^{-1}, 283.1 kJ/mol.

4-2) What would be the energy (per photon), wavelength, and frequency of the light emitted when a hydrogen atom falls from the 2nd excited state to the first excited state (see Figure 4-4)? Ans: 3.026×10^{-19} J, 656.5 nm, and 4.567×10^{14} s^{-1}.

Section 4.2: Absorption Spectroscopy

4-3) Assume that the area of a molecule which is responsible for the absorption of a photon is circular with a radius of 75 pm, what is the maximum value for ε for that molecule? Ans: 4.62×10^4 L/mol·cm.

4-4) A coloured glass light filter 2.00 mm thick is placed in a spectrophotometer and its %*T* measured as 8.7. The %*T* of a piece of glass of the same composition is measured as 79.4 in the same manner. What is the thickness of the glass? Ans: 0.189 mm.

4-5) A very simple spectrophotometer (without dark current or 100%*T* adjustments) is used to measure the concentration of a coloured material in a sample. With the light beam blocked, the meter (which has a linear scale) reads 5.3. With a blank in the light beam the reading is 83.5, with a standard of 5.00×10^{-5} M concentration the reading is 64.4, and with the sample the reading is 31.2. What is the sample concentration, assuming that Beer's law is obeyed? Ans: 1.97×10^{-4} M.

4-6) If the absorbance of a solution containing 1.00×10^{-5} M of a coloured copper complex is 0.286 in a 1.00 cm cell, what concentration of the coloured complex would be required to absorb exactly half the light passing through the same cell? Assume Beer's law is obeyed. Ans: 1.05×10^{-5} M.

4-7) The %*T* of a 1.00×10^{-5} M solution of a dye is measured as 28.6 in a 1.00 cm cell. 1.00 L of a 1.00×10^{-2} M solution of the same dye is poured into an irregularly shaped vessel containing clear water and the dye thoroughly mixed. A sample of the resulting solution reads 75.3 %*T* in the same 1.00 cm cell. What is the volume of the vessel? Ans: 4.41×10^{3} L.

4-8) The %*T* of a sample containing a coloured Fe(II) complex (in excess complexing reagent) is measured as 28.6. When 2.00 mL of a 1.00×10^{-4} M Fe(II) standard is mixed with 10.00 mL of the sample, the resulting solution reads 13.8 %*T* in the same cell. Assuming that Beer's law holds and that the iron complex is the only coloured material in the sample, calculate the concentration of Fe(II) in the original sample. Ans: 2.23×10^{-5} M.

4-9) Benzoic acid, which absorbs in the uv, can be analyzed in water samples by spectrophotometry. 10.00 mL of a water sample are diluted to 20.00 mL and read 56.3 %*T* in a spectrophotometer. 1.00 mL of a 5.00×10^{-5} M solution of benzoic acid is added to 10.00 mL of sample and diluted to 20.00 mL. This solution reads 51.2 %*T*. What is the concentration of benzoic acid in the sample?
Ans: 3.02×10^{-5} M.

4-10) Equilibrium constants can be determined using spectrophotometry. For example, a solution is prepared containing 2.00×10^{-2} F phenolphthalein (a Brønsted acid-base indicator). Three 1.00 mL portions of this solution are diluted to 100.0 mL with the following three solutions: (a) 0.100 F HCl, (b) 0.100 F NaOH, and (c) a buffer solution of pH 9.35 (where the formalities of both the buffer components are approximately 0.100). The absorbances of the three solutions as measured in a 1.00 cm cell are:

a) $A = 0.000$ (all indicator in the acid form).
b) $A = 0.732$ (all indicator in the base form)
c) $A = 0.242$

What is the pK_a of the indicator? Ans: 9.66

4-11) If a spectrophotometer exhibits only errors that are independent of *T*, and if that error is ±1.2 %*T*, what will be the relative error in concentration made by the instrument at 10 %*T*, 50 %*T*, and 90 %*T*? Ans: ±5.2, ±3.5, and ±13%.

4-12) Cu(II) and Ni(II) both form coloured complexes with an organic Lewis base reagent. The following data are available: at 450 nm ε for the Cu(II) complex is 1.53×10^{4} L/mol·cm and that of Ni(II) is 3.24×10^{3}, at 530 nm the values are 934 and 7.62×10^{3} respectively. An unknown containing both metal

ions gives a %*T* of 16.4 at both 450 and 530 nm in a 1.00 cm cell. Calculate the concentrations of the two metal ions in the unknown. Ans: [Cu(II)] = 3.03×10^{-5} M and [Ni(II)] = 9.93×10^{-5} M.

4-13) Sometimes colour forming reagents are so highly coloured themselves that they interfere in a spectrophotometric analysis. The reagent dithizone (a deep green colour) can be used in trace metal analysis. It is usually dissolved in a solvent such as dichloromethane and the metal ion reacts with this reagent and is extracted from the aqueous solution into the CH_2Cl_2, changing the colour of that layer. Assume the following:

	ε_{450}	ε_{550}
Coloured reagent	3.24×10^3	1.28×10^4
Coloured metal complex	1.07×10^4	2.44×10^3

If the absorbance of the coloured organic layer is measured as 0.447 at 450 nm and 0.731 at 550 nm, what is the concentration of the metal complex in the CH_2Cl_2 layer? Assume b = 1.00 cm. Ans: 2.60×10^{-5} M.

4-14) The following absorbance data are available for coloured complexes of Co(II) and Cr(III) with an uncoloured reagent in the same cell:

	450 nm	500 nm	550 nm	600 nm
1.00×10^{-4} M Co(II)	0.255	0.183	0.353	0.510
4.00×10^{-5} M Cr(III)	0.042	0.395	0.141	0.083
Mixture of Co(II) + Cr(III)	0.283	0.816	0.532	0.566

What are the concentrations of Co(II) and Cr(III) in the mixture? Ans: [Co(II)] = 8.37×10^{-5} M, [Cr(III)] = 6.71×10^{-5} M.

4-15) Consider a titration of the form: $U + T \rightarrow P_1 + P_2$. If the titrant (T) is the only coloured species in the reaction at the wavelength of interest, derive equations for the corrected absorbance before and after the equivalence point in the titration. Assume that ε_T = 1000 L/mol·cm, b = 1.00 cm, $[T]_{buret}$ = 0.00100 F, $[U]_o$ = 0.00100 F, V_U = 50.0 mL. What will be the corrected absorbance (calculated using only the equation you derived for the corrected absorbance after the end point) at 25.0, 50.0, and 75.0 mL of T added? Ans: -0.500, 0.000, 0.500.

Challenge Problems

4-16) Assume that a spectrophotometer set to a given wavelength actually passes two separate wavelengths, A and B, each with the same intensity. Secondly assume that the sample absorbs both wavelengths, but to different extents. If $\varepsilon_A = 9.00 \times 10^3$, $\varepsilon_B = 1.00 \times 10^4$, and b = 1.00 cm, what concentration of absorber will produce an absorbance of 0.100? Next calculate the deviation from Beer's law at absorbances of 1.00 and 2.00 using the following equation:

% deviation = ("ideal" conc.-actual conc.)(100)/("ideal" conc.)

The "ideal" concentration would be that calculated using Beer's law with an absorptivity which is the average of the two above. Ans: 1.0529×10^{-5} M, -0.32%, -0.64%.

4-17) A certain type of spectrophotometer exhibits one type of error which is constant with transmittance and which amounts to 0.25 %*T*, and a second type which is proportional to transmittance (relative error in *T* is constant) which amounts to 0.20 %. Assuming the worst case - that these errors are not random and that they are both in the same direction - at what transmittance will the total error be minimum? What will be the relative error in *C* at the minimum? Between what values of *T* will the relative error in *C* be equal to or less than 1.0 % (remember that if *dT* is positive, *dc/c* will be negative)? Ans: 0.291, -0.86 %, 0.493 and 0.144.

Section 4.3: Fluorescence

4-18) The fluorescence of a 1.00×10^{-7} F quinine sulfate solution was measured as 31.5 with a fluorometer while the background was 4.9. A water sample thought to contain quinine (when treated in the same manner as the standard and the blank) measured 52.9. Assuming that the concentration is low enough for fluorescence to be linear in concentration, calculate the concentration of quinine in the sample. Ans: 1.80×10^{-7} F.

4-19) The oxidized form of riboflavin does not fluoresce. 10.0 mL of a sample containing riboflavin is added to 10.0 mL of an oxidizing agent and the fluorescence is measured as 8.7. 10.0 mL of the sample are diluted to 20.0 mL and the fluorescence is measured as 38.6. Finally 10.0 mL of the sample plus 2.00 mL of a 1.00×10^{-6} F riboflavin solution are diluted to 20.0 mL and the fluorescence measured as 61.3. What is the concentration of riboflavin in the unknown (assuming fluorescence is linear in concentration)? Ans: $2.6_3 \times 10^{-7}$ F.

4-20) Assuming that a fluorescent analyte does not absorb any emitted light, that excitation light falls evenly on the entire width of the cell, and that the entire cell width can be "seen" by the detector, derive an equation which predicts the deviation from linearity of fluorescence as a function of *C* and ε, assuming that *b* = 1.00 cm. (HINT - use the ratio of observed fluorescence to "expected" or "ideal" fluorescence and the series expansion of the exponential retaining both the first and second order terms in *C*.) At what concentration will there be a 2% deviation (98% of the expected fluorescence is observed) if $\varepsilon = 1.00 \times 10^{4}$? Ans: 1.74×10^{-6} M.

4-21) The following data were obtained in a fluorescence analysis:

Solution	Fluorescence
blank	10.3
5.0×10^{-6} F	43.5
1.0×10^{-5} F	68.8
unknown	61.3

Assume only that the analyte does not absorb the fluorescent light and that the entire width of the cell is exposed to excitation radiation and is observed by the emission detector. What is the concentration of the analyte in the unknown? Ans: 8.4×10^{-6} F.

Section 4.4: Atomic Spectroscopy

4-22) A simple AA spectrometer is used to analyze copper in water samples. The blank provides a reading of 82.6, a 0.100 ppm Cu(II) solution gives a reading of 61.3, a sample gives a reading of 56.5

and the instrument reads 2.1 with the light beam blocked. What is the concentration of Cu(II) in the sample? Assume Beer's law holds. Ans: 0.128 ppm.

4-23) Solvent extraction, in which ions are extracted into an organic phase which is insoluble in water (*e.g.* nitrobenzene) , is used to overcome interferences and preconcentrate analytes in AA spectroscopy. 100.0 mL of an aqueous solution containing Cd(II) are extracted with 5.00 mL of nitrobenzene containing an extracting reagent and the absorbance of the organic layer is read as 0.341. A sample of nitrobenzene containing 0.200 ppm Cd(II) gives an absorbance of 0.463 under the same conditions. What is the concentration of Cd(II) in the water sample? (Assume Beer's law is obeyed.) Ans: 0.00737 ppm.

4-24) From the following data determine the concentration of Ba^{2+} in an unknown analyzed by AAS (assume linearity between *A* and concentration).

Solution	%*T*
sample	65.3
8.00 mL of sample + 2.00 mL of 2.00 ppm Ba^{2+}	53.6

Ans: 0.603 ppm.

4-25) The following data are taken on solutions containing both Cu(II) and Ag(I) using an AA spectrometer. These elements are very similar in their behaviour in AA spectroscopy, copper absorbing at 325 nm and silver at 338 nm.

[Cu(II)]	[Ag(I)]	A_{325}	A_{338}
2.00×10^{-6}	1.00×10^{-6}	0.136	0.193
4.00×10^{-6}	5.00×10^{-7}	0.273	0.098
6.00×10^{-6}	2.00×10^{-6}	0.405	0.388

Is the ratio A_{325}/A_{338} proportional to the ratio [Cu(II)]/[Ag(I)]? 2.00 mL of a solution containing 3.00×10^{-5} M Ag(I) are added to 10.0 mL of a sample containing copper but no silver. This solution is then treated in a way that changes the concentrations of its components (but not their ratios) in order to analyze for other elements. Then the values for A_{325} and A_{338} for this solution are determined to be 0.125 and 0.183 respectively. Using the ratio of these values and the proportionality constant calculate the concentration of Cu(II) in the original sample. Ans: 1.17×10^{-5} M.

4-26) Some materials prevent the atomization of certain metals in the flames normally used in AA spectroscopy. This interference is sometimes so quantitative that it can be used to determine the interferent. An example of such a system is the interference of phosphate on the atomic absorption of calcium. From the following data determine the concentration of phosphate in the sample.

Solution	A_{423}
2.00×10^{-6} M Ca^{2+}	0.538
2.00×10^{-6} M Ca + 1.00×10^{-6} M PO_4^{3-}	0.409
2.00×10^{-6} M Ca + 2.00×10^{-6} M PO_4^{3-}	0.280
10.0 mL sample + 10.0 mL 4.00×10^{-6} M Ca^{2+}	0.337

Ans: 3.12×10^{-6} M.

4-27) Uranium in well water is to be analyzed by ICP atomic emission at 358.4 nm. A solution of uranyl nitrate ($UO_2(NO_3)_2$) containing 50.0 ppb uranium gives an intensity of 185.3 arbitrary units. The well water sample reads 89.5 units and distilled water reads 20.5 units. What is the concentration of uranium in the well water? Ans: 20.9 ppb.

4-28) A saturated solution of CaF_2 (in equilibrium with solid CaF_2) produces an intensity reading of 116.1 on a flame photometer. A 5.00 ppm standard solution of Ca ion reads 66.8 and distilled water reads 8.7. From these data calculate the solubility product of CaF_2. Ans: 4.91×10^{-11}.