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White Paper

Understanding Spectroscopy

The Study of Energy and Light

One of the first instruments an analyst learns to operate in their career is some form of an energy-based detector. These types of detectors are often at the heart of other spectrometry and chromatography instruments. The basis for many GC and LC instruments is a spectrometry or spectrophotometry configuration in which energy (or light) plays an important role in analysis.

There is often a lot of confusion between the terms chromatography, spectroscopy, spectrometry, and spectrophotometry. These concepts are intrinsically linked but are still different approaches to looking at (or measuring) matter with light (or energy). The terms are all based around the Greek word origins for color (*chroma-*) and light (*photo-*) combined with the terms" 'to write' (*graphein*), 'to measure' (*metria*), 'to see' (*skopia*), or the Latin 'to look at' (*specere*). The different disciplines can be understood as a general sphere of study with levels of increasing specialty or specificity.

Spectrometry (the measurement of the interaction of energy and matter) is the basis for all of the other techniques. There are several types of spectrometry defined by their target and method of measurement. The most common analytical techniques found in the organic laboratory are mass spectrometry and spectroscopy.

Mass spectrometry (MS) measures the mass-to-charge ratio of charged particles which results in a mass spectrum (intensity vs. mass-to-charge plot). There are many types of mass spectrometry including the commonly used GC/MS, LC/MS and ICP-MS. These instruments provide large amounts of data which can be extremely important for many research and analytical processes. On the downside, the purchase, operation, and maintenance of these mass spectrometers can be costly, labor-intensive and time-consuming. Most mass spectroscopists see MS as a type of art-form with nuances requiring interpretation skills.

Spectroscopy is the study or measurement of the interaction of matter and electromagnetic radiation resulting in spectra (wavelength or frequency of the radiation). Sometimes spectroscopy is also described as the study of color from all bands of the electromagnetic radiation (EM) spectrum. Electromagnetic radiation is all waves in the electromagnetic field carrying electromagnetic radiation throughout space. Electromagnetic radiation is made up of oscillating waves of magnetic and electrical fields measured most often by frequency and wavelength.

Waves show regular repetitive changes in value where points in the wave are either in-phase (oscillating in unison) or out-of-phase (oscillating at different points not in unison). Wavelength is the distance measured between the nearest two points in phase with each other. These two adjacent peaks (or troughs) are said to be separated by a single wavelength (λ) while the distance from peak to trough is the amplitude (Figure 1).

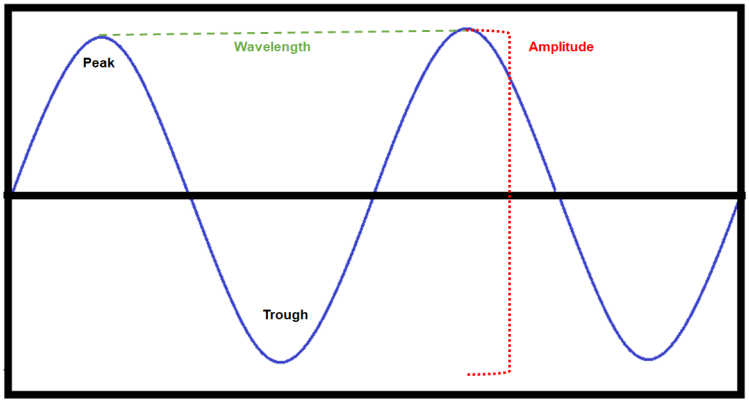

Figure 1. Wave Diagram

The number of peaks in a unit of time or space is called its frequency. When peaks, such as the wave discussed, are measured in time, it is referred to as a temporal frequency measured in hertz (Hz) (one event per second) and is the reciprocal of period (the duration of time for one cycle of an event such as the occurrence of peak and trough).

The Electromagnetic Radiation Spectrum

EM radiation spectrum encompasses a wide band of energy including radio waves, microwaves, infrared, visible light, ultraviolet, X-rays, and gamma rays (Figure 2). Radio waves at the end of the spectrum have the largest wavelength and the lowest frequency. The size of these radio waves is in the hundreds of meters, comparable with the size of buildings. On the other end of the range are gamma rays which are high frequency and so small they cannot be measured since the particles slip between the molecules of measurement devices (Figure 2).

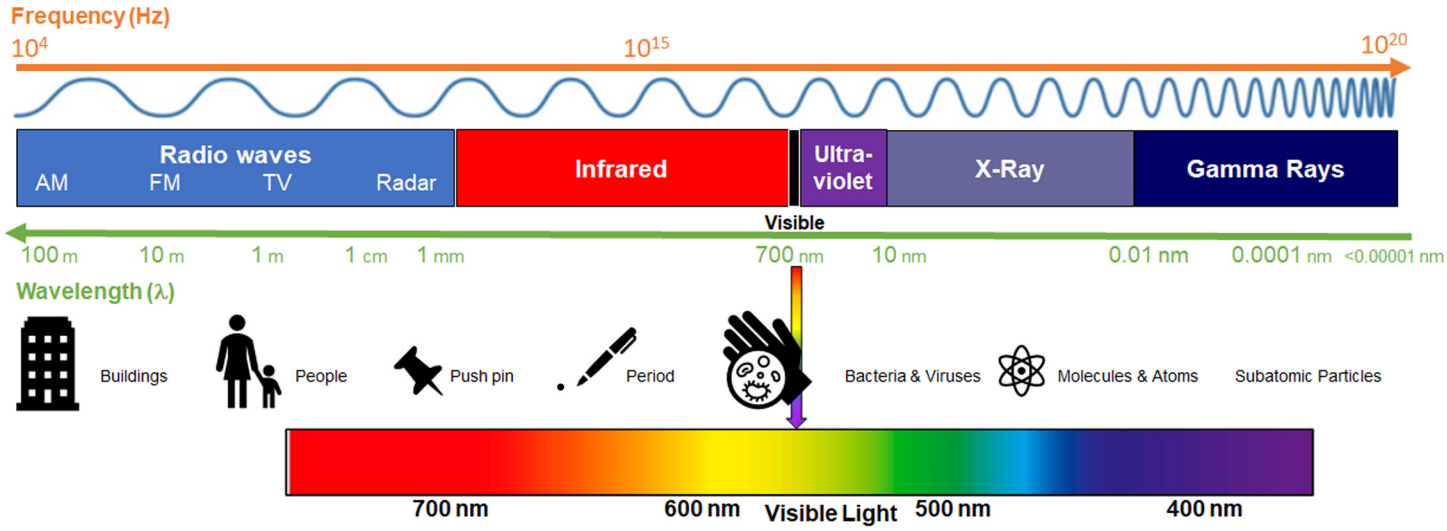

Figure 2. Electromagnetic Spectrum

Many laboratory analytical techniques are focused on a range of waves associated with light, from infrared to ultraviolet light, in the range of 100 nm to 1 mm. This energy encompasses the ultraviolet ranges, visible light and the infrared spectrum. Light outside of visible range for humans includes infrared light in the range upwards of 700 nm. IR light is divided into Infrared-A (700 – 1,400 nm), Infrared-B (1,400 – 3,000 nm) and Infrared-C (3,000 nm – 1 mm). These wavelengths of light have a longer spatial period for a periodic wave than visible light. IR radiation is used in a wide variety of applications including scientific and industrial equipment, law enforcement and medical devices. One of the common uses for IR is in the application of contactless thermometers during the COVID-19 pandemic.

On the other side of visible light is ultraviolet radiation which is also classified into three groupings: Ultraviolet-A (UVA) from 315 – 400 nm, Ultraviolet-B (UVB) from 280 – 315 nm, and Ultraviolet-C (UVC) from 100 – 280 nm. UVA was frequently used in artificial tanning before it was discovered to cause formation of free radicals and reactive oxygen. UVB is absorbed, to a large extent (along with UVC), by the Earth's atmosphere. UVB and UVC cause the reaction leading to the production of ozone. UVB is a double-edged sword, it is needed for the production of ozone and the production of vitamin D in the body, but it is also the leading cause for sunburn and DNA damage leading to cancer. UVB is very photoreactive and can cause unwanted DNA changes in the photoreactive cells of the epidermis, such as melanocytes and basal cells. UVA has the longest wavelength and penetrates beyond the skin's hypodermis into the deeper dermis and can also change the DNA of skin cells, but not the same degree as UVB radiation.

Visible Light and Color

Most animals, including human beings, can only perceive light within the range of 400 – 700 nanometers, which also gives us the perception of colors when they are absorbed by objects and reflect back as the opposite color (Table 1 and Figure 3). We see the color red when light of many spectrum hit something like an apple and all of the other wavelengths of visible light are absorbed while the reds are reflected back for the human eye to see.

Table 1. Wavelengths of Color

Wavelength (nm)	Color Absorbed	Color Reflected/Observed
400-435	Violet	Yellow-Green
435-480	Blue	Yellow
480-490	Green-Blue	Orange
490-500	Blue-Green	Red
500-560	Green	Purple
560-580	Yellow-Green	Violet
580-595	Yellow	Blue
595-605	Orange	Green-Blue
605-700	Red	Blue-Green

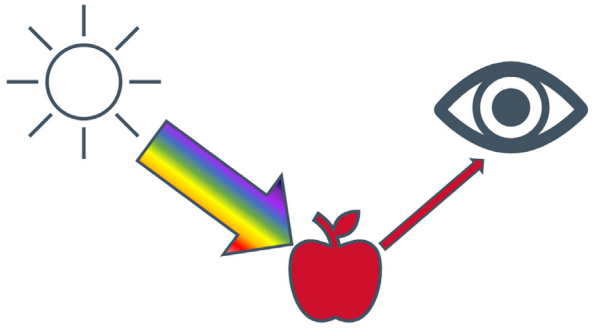

Figure 3. Example of Absorption and Reflection of Light

Energy and Matter Interactions

There are numerous interactions between EM and matter. These include some of the most commonly used interactions in instrumentation including adsorption, transmission/refraction, reflection, and emission. Absorption occurs when matter transforms EM energy into internal energy (such as thermal energy) through an absorber. Transmission and refraction are a function of the amount, wavelength and angle of which energy (or light) pass through matter. Reflection is the measurement of the amount of energy reflected matter, and emission is the change in energy state as the incident energy passes through matter. All of these processes become the basis for some form of detection or spectroscopy measurement technique.

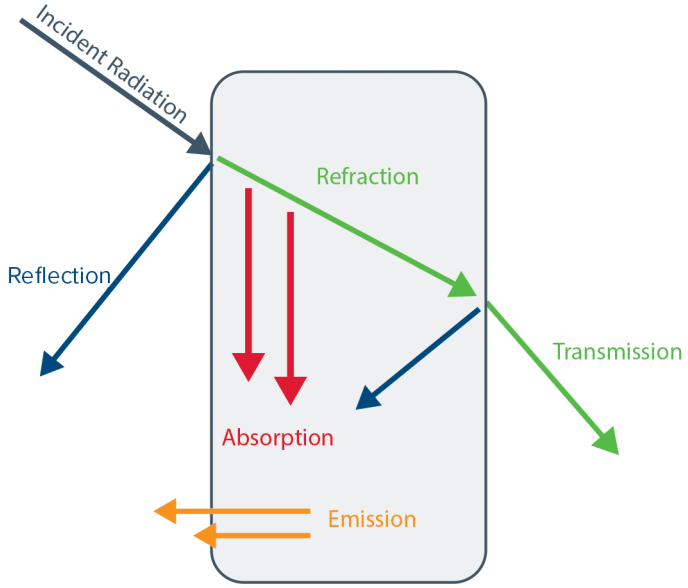

Figure 4. Basic Types of Energy and Matter Interaction in Spectroscopy

Other types of spectroscopy interactions include elastic scattering (similar to reflection) where the incident beam is scattered within the target material rather than just reflected. Inelastic scattering also involves the measurement of scattering but, as the function of a change in wavelength. Impedance is the slowing of the transmission of energy. Resonance spectroscopy is characterized by radiant energy being a radiating field between quantum states of the material. Finally, there is nuclear spectroscopy that utilizes the nuclei to determine the properties of matter.

Spectroscopy techniques are divided into atomic and molecular spectroscopy depending on the target to be measured and the material being tested. **Atomic spectroscopy** studies the energy and matter interactions between atoms. Most of the atomic spectroscopy techniques are applied to the study of elemental composition. The instruments under atomic spectroscopy include atomic absorption (AA), X-ray fluorescence (XRF), and inductively coupled plasma (ICP). **Molecular spectroscopy** studies the interaction of energy and matter between molecules. It is most often found in techniques measuring organic molecules and includes instruments such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and UV and visible light spectroscopy.

Spectrometry instruments are often further divided and defined by the type of interactions they produce and the type of energy that is measured, like X-ray fluorescence (XRF) which measures X-rays and the amount of fluorescent energy a material emits during exposure. Some techniques focus in a narrow band of interactions (such as emission only) or a small part of the EM spectrum (like X-rays), while other techniques monitor an array of interactions and wavelengths on the spectrum.

When techniques using mostly the basic absorption, reflection and transmission properties focus on the energy bands included in the wavelengths of 'light'; they are frequently referred to as spectrophotometers. The basic pathway for a spectrophotometer contains an energy or light source which is then sometimes split or isolated by a filter or monochromator before encountering a sample. The energy interacts with the sample and the energy which is transmitted, absorbed, emitted, and/or reflected is measured by a detector (Figure 5).

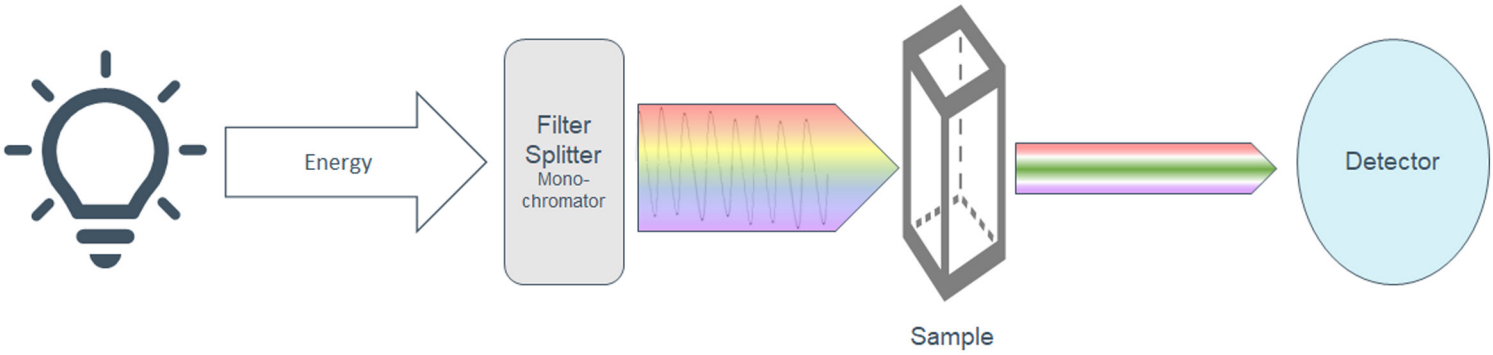

Figure 5. General Spectrophotometer Pathway

Spectrophotometer Configurations

Spectrophotometers are a common, easy-to-use spectrometer found in many organic analytical chemistry laboratories. They often work on the simple principle of comparing the absorbance or transmission of light through a liquid sample compared to a blank or a standard material. The difference or change from the blank or standard material is used to calculate concentration or a number of other results.

The most widely used form of spectrophotometers are UV or UV/VIS spectrometers operating in the UV and visible wavelengths of light from 180 – 800 nm. There are several options and configurations for spectrophotometers including number of pathways (i.e. beams) detectable and the types of modes of detection performed.

Spectrophotometers are characterized as either single beam, containing only one pathway from source to detector and therefore only having one sample pathway (Figure 5), or a double beam (split) system which splits the energy source prior to interacting with the sample and diverts to a second sample cuvette that most often contains a blank reference standard or sample (Figure 6).

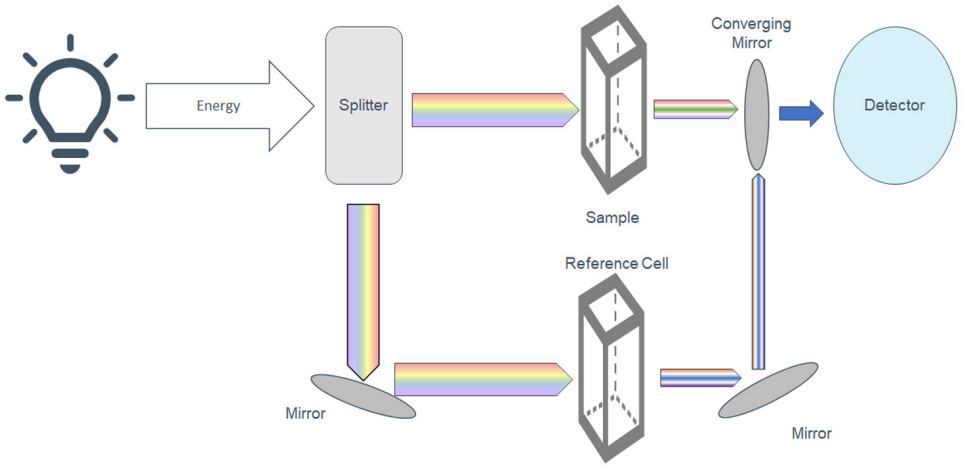

Figure 6. General Schematic of Double Beam, Alternating In-Time Spectrophotometer

There are two basic types of double beam configurations. Alternating in-time configurations have a series of converging mirrors that bring the beam back to one detector. The beam is directed at alternating times between the two cuvettes and the detection occurs alternately as well. Simultaneous in-time configurations split the beam between the two cuvettes but do not direct the resulting beams to a single detector, but in fact have multiple detectors which can operate simultaneously during scans.

Some spectrophotometers have preprogrammed settings and only allow for the monitoring of selected or a single wavelength (fixed wavelength). This configuration usually employs some filter or slit that can be programmed to allow only single wavelengths to be exposed to the sample. In contrast, scanning and array spectrophotometers allow for an entire range, multiple sets of wavelengths or scanning subsequent wavelengths along an entire range of wavelengths. A scanning spectrometer uses a tunable monochromator to isolate individual wavelengths of light from the incident beam before interacting with the sample. An array spectrophotometer allows for the simultaneous scanning of a range and has no filter or monochromator, however, there is often a focusing slit to confine the incident beam. This configuration allows the entire focused incident beam to interact with the sample and the data from individual wavelengths occur at the detector (known as reverse options) (Figure 7).

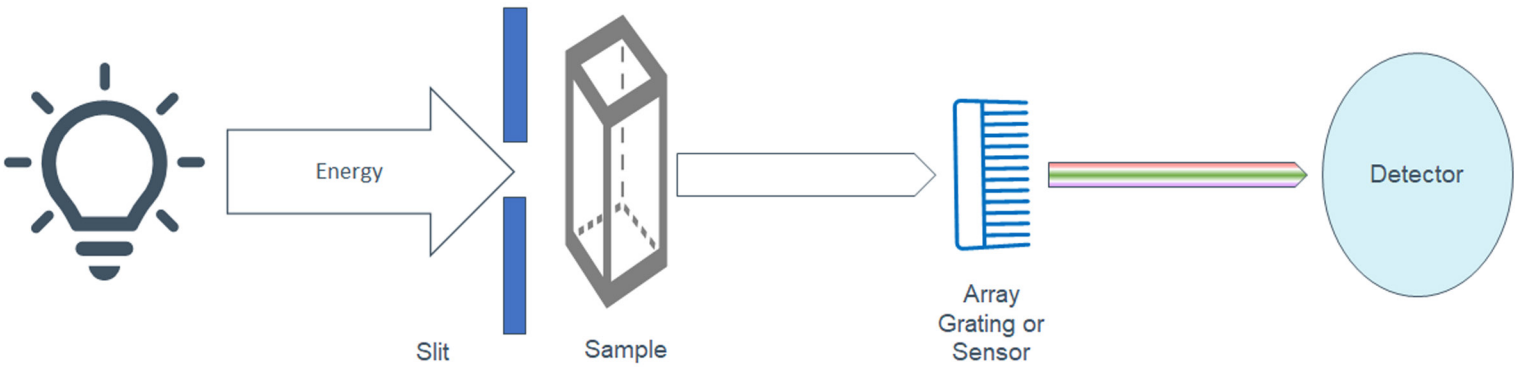

Figure 7. Reverse Optical Array Spectrometer General Schematic

Spectrophotometry Lamps

The incident energy or light is provided by several different types of lamps including halogen, xenon, deuterium, and light-emitting diodes (LED). Halogen lamps (also known as tungsten or quartz lamps) are similar to incandescent light bulbs and cover the wavelengths of visible light from about 320 – 1,100 nm. In this lamp, the filament heats up and emits light when a current flows through it. The bulb is filled with inert gas to prevent evaporation of the tungsten filament. A halide is also contained in the lamp to create the halogen cycle to return evaporated tungsten to the filament and reduce blackening of the bulb. Instruments with only halogen lamps can only detect wavelengths of visible light. Generally, halogen lamps last about 2,000 hours and are comparatively low cost.

Deuterium (D2) lamps operate in the range of 190 – 370 nm and are often paired with halogen lamps to cover the UV/VIS spectrum. D2 lamps operate at high temperatures and require specialized quartz housings which can increase cost. A D2 lamp is a discharge light source with deuterium sealed in a bulb. A hot cathode is used to deliver a steady arc discharge. These lamps require a large power supply and adds to the cost. D2 lamps last about 1,000+ hours.

Xenon lamps are discharge lamps and high energy sources just like the D2 lamp, but xenon lamps cover the entire UV/VIS range and more from 185 – 2,000 nm and eliminate the need for two types of lamps. Xenon gas is sealed in the bulb with a tungsten electrode. The lamp operates at 80 Hz which can prolong its life, but comes with increased costs higher than halogen or deuterium lamps. LED lamps produce single wavelengths of light with only small variations in bandwidth. The positive side means no monochromator is needed for some spectrophotometers.

Each type of lamp has its own benefits and drawbacks including range, cost and lamp life. Some technological or programming advances for spectrophotometers can increase lamp life by limiting lamp emissions to set time periods. One feature called press-to-read (PTR) ensures the lamp is only 'on' when needed to increase lamp life.

Spectrophotometry Measurement Modes and Calculations

In all of the configurations of spectrophotometers, there are often multiple modes in which a system can be operated. Many multi-mode instruments can operate in standard fixed wavelength or multi-wavelength modes and some in scanning modes as well. The data can then be used for different types of calculations such as for transmittance, absorbance, concentration, and kinetics.

Transmittance (T) is the calculation of the difference of intensity (I_o) energy from the incident beam to the resulting beam (I) (Figure 8). Transmittance is usually expressed in units of percent.

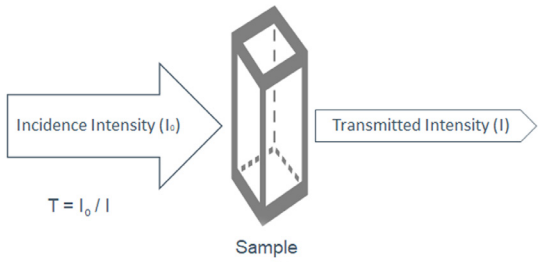

Figure 8. Transmittance Equation and Diagram

The next type of measurement mode is the negative log of transmittance called absorbance (1).

$$A = -\log(T) \quad \text{(1) Absorbance}$$

When a sample in solution is placed in a transparent cuvette, the light intensity is found to be proportional to the sample concentration and the length of the pathway of the cuvette (Figure 9). This is called Beer Lambert Law.

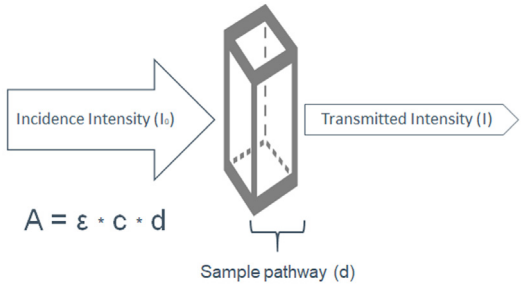

Figure 9. Beer Lambert Law Diagram and Equation

A standard type of result is obtained by calculating concentration with either a factor or a standard curve. In this type of calculation, the concentration of a sample is determined by multiplying an absorbance (Abs) value by a specific factor. This type of calculation used Beer Lambert Law where the concentration is proportional to the absorbance (2).

$$c = \frac{A}{\varepsilon * d}$$

(2) Beer Lambert Law

c = concentration, A = Absorbance, d = pathlength of cuvette (cm) and ε = extinction coefficient.

The extinction coefficient (ε) is the characteristic of a substance that tells how much light is absorbed at a particular wavelength per concentration, molarity or pathlength. The extinction coefficient is sample specific and describes how much the sample can absorb in L/(cm*mol) or mL/(cm*g). If the factor is now known, then the concentration of a sample can be determined by standard curve where known concentration standards are measured, and a standard curve is created.

Some other modes of data include kinetic measurements where the absorbance of a sample is measured over time for changes. The change in absorbance is plotted, giving rise to details regarding reaction rates. In the case of these types of measurements, internal standards can also be added to compensate for any losses or variations and correct for bias.

Other Important Considerations

There are a number of other specifications such as accuracy, resolution and bandwidth which should be considered in any spectrometers. In chromatography systems these terms refer to the size and retention time of peaks, but in spectrometers it refers to the resolution and accuracy of the measurement around each wavelength being measured.

Sometimes a spectrophotometric system's function is enhanced by the accessories which aid measurement including high throughput sampling systems like multi cuvette holders and changers, and sippers (pumping systems allowing for either very accurate or continuous sample introduction), or temperature controlled accessories for temperature sensitive samples.

Most basic spectroscopy systems are equipped to perform routine analysis and experiments. Higher end instruments can provide more functionality but increase cost and the learning curve for operation. The selection of a spectrophotometry system ultimately is a trade-off of functionality, target, resolution, and price.