Idaho State Police
Forensic Laboratory Training Manual
Spectroscopy

1.0.0 INTRODUCTION TO SPECTROSCOPY

The term *spectroscope* derives from two root words: the Latin word spectrum meaning image, and the Greek word *skopein*, to view (e.g. micro*scope*, tele*scope*, etc.). So a spectroscope is an instrument that permits visual observation of spectra. Instruments that record a spectral image are commonly called spectrographs. If a photoelectric cell is used to measure how much of each color appears the instrument is called a spectrometer (from the Greek *metron*, measure). Since a device for measuring the intensity of radiation is called a photometer, the final combined term is *spectrophotometer*, or recording spectrophotometer.

Every atom, ion or molecule has a unique and characteristic interaction with electromagnetic radiation. The way in which radiation interacts with matter can be used to gain information about the matter. Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when molecules, atoms or ions of a sample move from one energy state to another.

The interaction of matter and radiation takes place throughout the entire spectrum of electromagnetic radiation. The nature of the radiation throughout this spectrum is the same; the only difference is in the frequency and wavelength of the radiation and the effects the radiation produces on matter. Radiation is photons moving at or near the speed of light. The type of interaction a photon has with matter is very much dependant on that photon's energy. The long wavelength – low frequency photons in the radio frequency range have only sufficient energy to cause a reorientation of the nuclear spin states of substances. Photons in the infrared region, cause changes in the vibrational and rotational energies of molecules. The high frequency, short wavelength photons in the X-ray region have sufficient energy to eject inner shell electrons from atoms. Figure 1 depicts the electromagnetic spectrum and shows the different regions of this spectrum as well as the changes to atoms or molecules caused by the radiation.

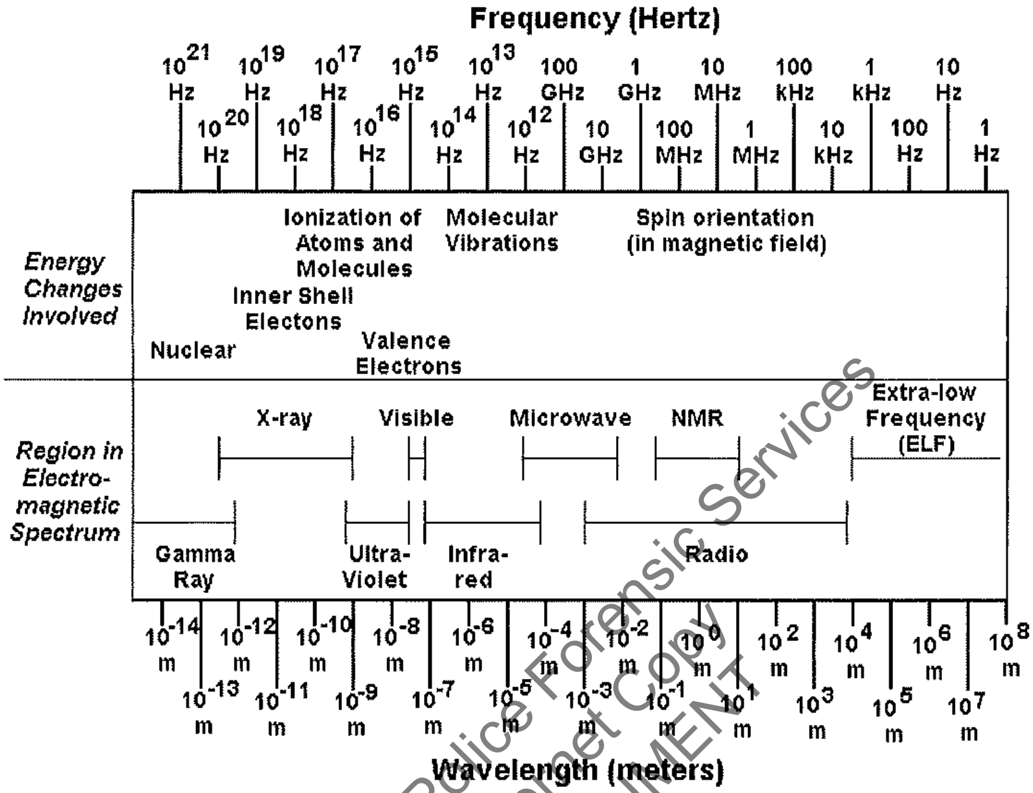

Figure 1 - The Electromagnetic Spectrum

In general, two types of measurements are taken with spectrometers – absorbance and emission.

With absorbance, radiation is passed through the sample of interest, and the amount of the radiation absorbed at different wavelengths is determined. Infrared (IR), ultraviolet/visible (UV/Vis) and atomic absorbance (AA) are common examples of absorbance spectrometry.

In emission spectrometry, the molecules or atoms in the sample of interest are bombarded with sufficient energy to raise them to an excited state. As they fall back to their normal state, they emit characteristic radiation. X-ray fluorescence, ultraviolet fluorescence and atomic emission (such as flame emission and inductively coupled argon plasma (ICP)) are examples of emission spectrometry.

All spectrometers need an energy source – either to excite the sample into emitting radiation, or as a source of radiation for the sample to absorb. All spectrometers also need a way of discriminating between different wavelengths that have interacted with the sample. Instruments dealing with different regions of the electromagnetic spectrum take different approaches. Many instruments use monochromators, which disperse and separate the different wavelengths of radiation. Some instruments use filters to remove the wavelengths that are not of interest. Other instruments use energy discriminating detectors. The specific sources and methods for discriminating between the wavelengths of radiation will be explained in detail later in the reading material.

A photon has the properties of a microscopic particle of definite energy and at the same time has properties of a wave extending over a broad area of space. When a photon passes a particular region of space, the electric field in that region oscillates with the frequency of that photon. Figure 2 gives a graphical representation of this oscillation.

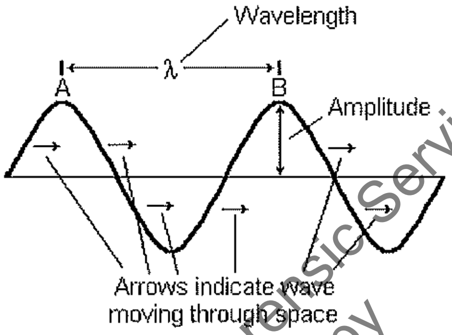

Figure 2

The number of waves passing a fixed point in one second is the frequency (□) of the radiation. Frequency is usually expressed in hertz (Hz) that is, cycles per second. The distance between successive maxima (points A and B on Figure 2) is the wavelength (□). The units commonly used for describing wavelength differ considerably in the various spectral regions. For example, the nanometer, nm (10^{-9} m), is employed with visible and ultraviolet radiation and the micrometer, □m (10^{-6} m), is used for the infrared region. The relationship of frequency to wavelength is given in Equation 1 where *c* is the speed of light in a vacuum. The true value for the speed of light in a vacuum is 2.99792×10^{8} m/sec but is usually rounded off to 3.00×10^{8} m/sec (3.00×10^{10} **cm**/sec). In air the speed of light is within 0.01% of the speed of light in a vacuum.

$$\square\,\square c/\square$$

Equation 1

Sometimes the wave number is used instead of the frequency. Wave numbers are the number of waves per centimeter and are expressed in units' cm^{-1}. As shown in Equation 2, wave numbers are equal to the reciprocal of wavelength and also equal to frequency divided by the speed of light.

$$\bar{v} = 1/\lambda(cm) = v/c = v/3x10^{10}(cm/s)$$

Equation 2

The energy of a photon, *E*, is given in Equation 3, where *h* is Planck's constant (6.6262×10^{-34} J/Hz).

$$\square E \;\square\; h\square\,\square hc/\square$$

Equation 3

As can be seen from Equation 3, the energy of a photon is directly proportional to its frequency, and inversely proportional to its wavelength. In other words, the energy of a photon increases as its wavelength decreases and frequency increases.

While frequency depends only on the source of radiation and remains constant for a given radiation, the velocity at which light moves through a medium is dependent upon both the composition of the medium and the frequency of the radiation. This leads to a phenomenon known as refraction, in which light changes direction when going from one medium to another in which the speed of light is different (see Figure 3).

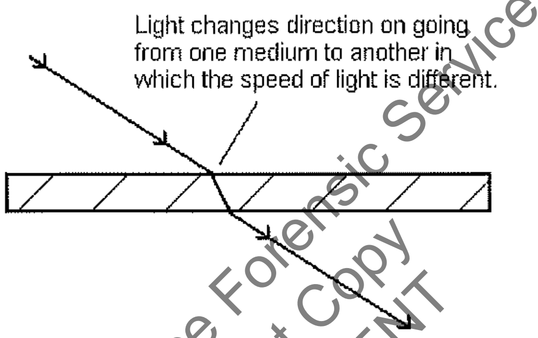

Figure 3 - Refraction

Because the speed of light in different media varies depending on the frequency of light, the amount of refraction also varies depending on the frequency of light. Shorter wavelengths are bent more than longer ones. These differences in refraction can be used to disperse, or separate, different frequencies of light (see Figure 4).

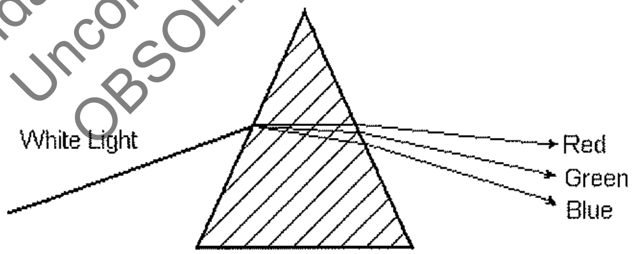

Figure 4 - Dispersion by a Prism

Prisms can be used to disperse ultraviolet, visible and infrared radiation. The material the prism is made of will differ depending upon the wavelength region. Prisms are rarely used in modern monochromators. They have been replaced by diffraction gratings, which consist of parallel grooves cut into a surface, ranging in density from 10,000 to 61,000 grooves per inch. Diffraction gratings offer several advantages as dispersing elements. Perhaps the most important is the wavelength independence of dispersion that makes the design of a monochromator considerably simpler. With prisms, short wavelengths are not dispersed as much as long wavelengths, so other parts of the monochromator have to compensate as a spectral range is scanned. Gratings also provide a means of dispersing radiation in the far ultraviolet and far infrared regions where absorption prevents the use of prisms. The chief disadvantages of gratings are that they produce more stray light and they pass higher order spectra ("overtones"). Gratings are still used in ultraviolet spectrometers, however in modern infrared spectrometers they have been largely replaced by interferometers. The function of monochromators and interferometers will be covered in the reading material in the Ultraviolet/Visible and Infrared Spectroscopy sections of this manual.

Three important radiation phenomena to consider are diffraction, reflection and scattering.

Diffraction is the departure from the rectilinear propagation of light rays and occurs when light rays encounter some sort of barrier. Diffraction occurs when the dimensions of the barrier or the dimensions of the openings in the barrier are comparable in size to the wavelength of light involved. When light is passed through a very narrow slit, it is "diffracted" or spread out in a manner somewhat analogous to water under pressure spraying out of a narrow nozzle. (See Figure 5). As narrow slits are often used in monochromators, diffraction must be taken into account.

Figure 5 - Diffraction

Diffraction can also be caused by a well-defined straight edge, such as the lines on a diffraction grating.

<u>Reflection</u> – There are two types of reflection. Specular reflection, or gloss, may be defined as the degree to which a surface possesses the light-reflecting property of a perfect mirror. With mirror like reflection, the angle of reflection equals the angle of incidence. With specular reflectance, a "mirror image" is usually produced. Diffuse reflection is seen with dull surfaces. The radiation is reflected from the sample surface through a wide range of angles. (See Figure 6.) A perfectly diffuse reflecting surface (matte), even under unidirectional illumination, has a constant luminance regardless of the angle from which it is viewed. With diffuse reflectance, no "mirror image" is produced.

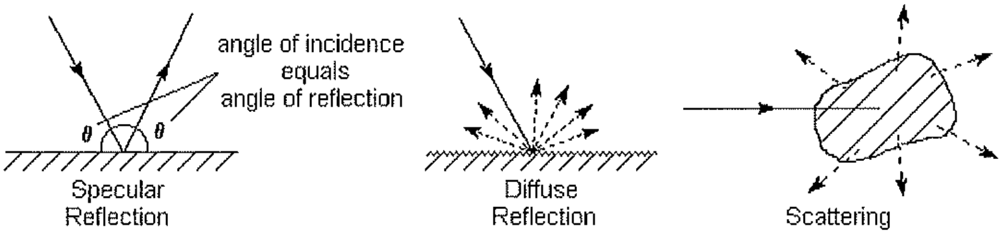

Figure 6

Reflection occurs whenever a light ray encounters a boundary between two media. These encounters are repeated over and over again in granular or fibrous structures where a light beam will encounter a new interface every few millionths of a centimeter. These repeated encounters result in thorough diffusion such that the surface tends to appear uniformly bright in all directions: this is the diffuse component that is responsible for color where color exists. Particle size plays an important role. Whiteness and reflectance gain as the diameter is reduced to about one-half of the wavelength of the incident light. At very small particle diameters, less than one-fourth the light wavelength, scattering takes over and diffuse reflectance falls of. As the particle size becomes very small in relation to the wavelength, scattering becomes less and less of a problem. Reflection and scattering are problems with turbid and opaque samples.

The amount of radiation a sample absorbs cannot be directly measured. It must be determined indirectly. What can be measured is the amount of radiation a sample allows to transmit through it. Transmittance (T) is defined in Equation 4:

$$\text{Transmittance (T)} = \frac{I}{I_0}$$

Equation 4

Where I_0 is the intensity of the radiant energy striking the sample and I is the intensity of the radiation emerging from the sample.

Absorbance is related to transmittance as shown in Equation 5.

$$\text{Absorbance (A)} = -\log T = \log_{10}\frac{1}{T} = \log_{10}\frac{I_0}{I}$$

Equation 5

Infrared spectrums are usually displayed as % transmittance verses wavelength while Ultraviolet/Visible spectra are generally displayed as absorbance verses wavelength. This is probably due to the fact that UV/Vis is more commonly used as a quantitative technique and it is easier to relate absorbance to concentration. Quantitation will be discussed further in the Ultraviolet/Visible Spectroscopy section.

2.0.0 QUESTIONS

1. How is the energy of a photon related to its wavelength and frequency?
2. When the bottom half of a stick has been placed in water, why does it appear that the stick bends at the point it enters the water?
3. What is frequency? Under what name is frequency usually expressed as? What are the units of this frequency?
4. What is a wave number and what are wave numbers units?
5. How can wavelength be converted to wave numbers?
6. How is frequency related to wavelength?