

CHAPTER 1

FUNDAMENTALS OF SPECTROSCOPY

INTRODUCTION

Spectroscopy is a powerful tool for studying biological systems. It often provides a convenient method for analysis of individual components in a biological system such as proteins, nucleic acids, and metabolites. It can also provide detailed information about the structure and mechanism of action of molecules. In order to obtain the maximum benefit from this tool and to use it properly, a basic understanding of spectroscopy is necessary. This includes a knowledge of the fundamentals of spectroscopic phenomena, as well as of the instrumentation currently available. A detailed understanding involves complex theory, but a grasp of the important concepts and their application can be obtained without resorting to advanced mathematics and theory. We will attempt to do this by emphasizing the physical ideas associated with spectral phenomena and utilizing a few of the concepts and results from molecular theory.

Very simply stated, spectroscopy is the study of the interaction of radiation with matter. Radiation is characterized by its energy, E , which is linked to the frequency, ν , or wavelength, λ , of the radiation by the familiar Planck relationship:

$$E = h\nu = hc/\lambda \quad (1-1)$$

where c is the speed of light, 2.998×10^{10} cm/s (2.998×10^8 m/s), and h is Planck's constant, 6.625×10^{-27} erg-s (6.625×10^{-34} J-s). Note that $\lambda\nu = c$.

Radiation can be envisaged as an electromagnetic sine wave that contains both electric and magnetic components, as shown in Figure 1-1. As shown in the figure, the electric component of the wave is perpendicular to the magnetic component. Also shown is the relationship between the sine wave and the wavelength of the light. The useful wavelength of radiation for spectroscopy extends from x-rays, $\lambda \sim 1\text{--}100$ nm, to microwaves, $\lambda \sim 10^5\text{--}10^6$ nm. For biology, the most useful radiation for spectroscopy is in the ultraviolet and visible region of the spectrum. The entire useful spectrum is shown in Figure 1-2, along with the common names for the various regions of the spectrum. If

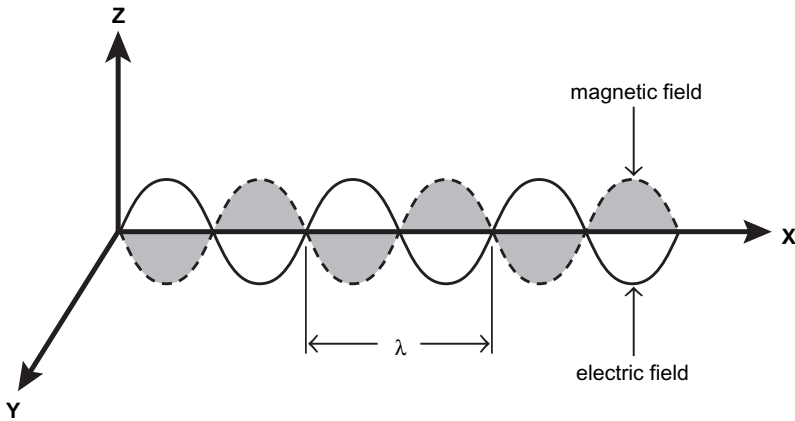


Figure 1-1. Schematic representation of an electromagnetic sine wave. The electric field is in the xz plane and the magnetic field in the xy plane. The electric and magnetic fields are perpendicular to each other at all times. The wavelength, λ , is the distance required for the wave to go through a complete cycle.

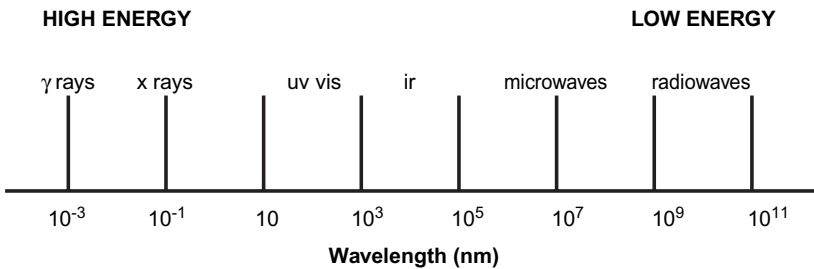


Figure 1-2. Schematic representation of the wavelengths associated with electromagnetic radiation. The wavelengths, in nanometers, span 14 orders of magnitude. The common names of the various regions also are indicated approximately (uv is ultraviolet; vis is visible; and ir is infrared).

radiation is envisaged as both an electric and magnetic wave, then its interactions with matter can be considered as electromagnetic phenomena, due to the fact that matter is made up of positive and negative charges. We will not be concerned with the details of this interaction, which falls into the domain of quantum mechanics. However, a few of the basic concepts of quantum mechanics are essential for understanding spectroscopy.

QUANTUM MECHANICS

Quantum mechanics was developed because of the failure of Newtonian mechanics to explain experimental results that emerged at the beginning of the 20th century. For example, for certain metals (e.g., Na), electrons are emitted when light is absorbed. This *photoelectric* effect has several nonclassical characteristics. First, for light of a given frequency, the kinetic energy of the electrons emitted is independent of the light intensity. The number of electrons produced is proportional to the light intensity, but all of the electrons have the same kinetic energy. Second, the kinetic energy of the photoelectron is zero until a threshold energy is reached, and then the kinetic energy becomes proportional to the frequency. This behavior is shown schematically in Figure 1-3, where the kinetic energy of the electrons is shown as a function

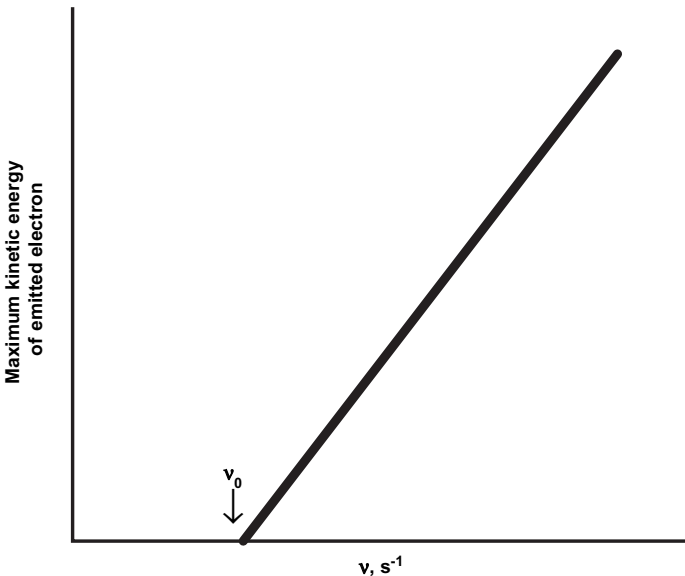


Figure 1-3. Schematic representation of the photoelectric effect. The maximum kinetic energy of an electron emitted from a metal surface when it is illuminated with light of frequency ν is shown. The frequency at which electrons are no longer emitted determines the work function, $h\nu_0$, and the slope of the line is Planck's constant (Eq. 1-2).

of the frequency of the radiation. An explanation of these phenomena was proposed by Einstein, who, following Planck, postulated that energy is absorbed only in discrete amounts of energy, $h\nu$. A photon of energy $h\nu$ has the possibility of ejecting an electron, but a minimum energy is necessary. Therefore,

$$\text{Kinetic Energy} = h\nu - h\nu_0 \quad (1-2)$$

where $h\nu_0$ is the *work function* characteristic of the metal. This predicts that altering the light intensity would affect only the number of photoelectrons and not the kinetic energy. Furthermore, the slope of the experimental plot (Fig. 1-3) is h .

This explanation of the photoelectric effect postulates that light is corpuscular and consists of discrete photons characterized by a specific frequency. How can this be reconciled with the well-known wave description of light briefly discussed above? The answer is that both descriptions are correct—light can be envisioned either as discrete photons or a continuous wave. This wave-particle duality is a fundamental part of quantum mechanics. Both descriptions are correct, but one of them may more easily explain a given experimental situation.

About this point in history, de Broglie suggested this duality is applicable to matter also, so that matter can be described as particles or waves. For light, the energy is equal to the momentum, p , times the velocity of light, and by Einstein's postulate is also equal to $h\nu$.

$$E = h\nu = pc \quad (1-3)$$

Furthermore, since $\lambda\nu = c$, $p = h/\lambda$. For macroscopic objects, $p = mv$, where v is the velocity and m is the mass. In this case, $\lambda = h/(mv)$, the de Broglie wavelength. These fundamental relationships have been verified for matter by several experiments such as the diffraction of electrons by crystals. The postulate of de Broglie can be extended to derive an important result of quantum mechanics developed by Heisenberg in 1927, namely the uncertainty principle:

$$\Delta p \Delta x \geq h/(2\pi) \quad (1-4)$$

In this equation, Δp represents the uncertainty in the momentum and Δx the uncertainty in the position. The uncertainty principle means that it is not possible to determine the precise values of the momentum, p , and the position, x . The more precisely one of these variables is known, the less precisely the other variable is known. This has no practical consequences for macroscopic systems but is crucial for the consideration of systems at the atomic level. For example, if a ball weighing 100 grams moves at a velocity of 100 miles per hour (a good tennis serve), an uncertainty of 1 mile per hour in the speed gives $\Delta p \sim 4.4 \times$

10^{-2} kg m/sec and $\Delta x \sim 2 \times 10^{-33}$ m. We are unlikely to worry about this uncertainty! On the other hand, if an electron (mass = 9×10^{-28} g) has an uncertainty in its velocity of 1×10^8 cm/sec, the uncertainty in the position is about 1 \AA , a large distance in terms of atomic dimensions. As we will see later, quantum mechanics has an alternative way of defining the position of an electron.

A second puzzling aspect of experimental physics in the late 1800s and early 1900s was found in the study of atomic spectra. Contrary to the predictions of classical mechanics, discrete lines at specific frequencies were observed when atomic gases at high temperatures emitted radiation. This can only be understood by the postulation of discrete energy levels for electrons. This was first explained by the famous Bohr atom, but this model was found to have shortcomings, and the final resolution of the problem occurred only when quantum mechanics was developed by Schrödinger and Heisenberg in the late 1920s. We will only consider the development by Schrödinger, which is somewhat less complex than that of Heisenberg.

Schrödinger postulated that all matter can be described as a wave and developed a differential equation that can be solved to determine the properties of a system. Basically, this differential equation contains two important variables, the kinetic energy and the potential energy. Both of these are well-known concepts from classical mechanics, but they are redefined in the development of quantum mechanics. If the wave equation is solved for specific systems, it fully explains the previously puzzling results. Energy is quantized, so discrete energy levels are obtained. Furthermore, a consequence of quantum mechanics is that the position of a particle can never be completely specified. Instead, the probability of finding a particle in a specific location can be determined, and the average position of a particle can be calculated. This probabilistic view of matter is in contrast to the deterministic character of Newtonian mechanics and has sparked considerable philosophic debate. In fact, Einstein apparently never fully accepted this probabilistic view of nature. In addition to the above concepts, quantum mechanics also permits quantitative calculations of the interaction of radiation with matter. The result is the specification of rules that ultimately determine what is observed experimentally. We will make use of these rules without considering the details of their origin, but it is important to remember that they stem from detailed quantum mechanical calculations.

PARTICLE IN A BOX

As an example of a simple quantum mechanical result that leads to quantization of energy levels, we consider a particle of mass m moving back and forth in a one-dimensional box of length L . This actually has some practical application. It is a good model for the movement of pi electrons that are delocalized over a large part of a molecule, for example, biological molecules such as carotenoids, hemes, and chlorophyll. This is not an ordinary box because inside

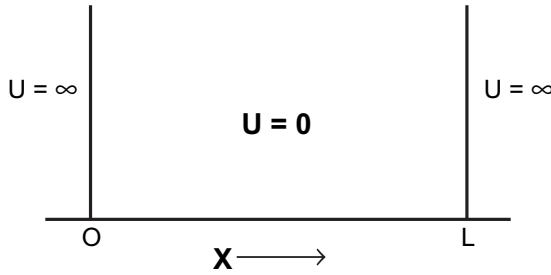


Figure 1-4. Quantum mechanical model for a particle in a one-dimensional box of length L . The particle is confined to the box by setting the potential energy equal to 0 inside the box and to ∞ outside of the box.

the box, the potential energy of the system is 0, whereas outside of the box, the potential energy is infinite. This is depicted in Figure 1-4. The Schrödinger equation in one dimension is

$$-\frac{\hbar^2}{8m\pi^2} \frac{d^2\Psi_n}{dx^2} + U = E_n\Psi_n \quad (1-5)$$

where Ψ_n is the wave function, x is the position coordinate, U is the potential energy, and E_n is the energy associated with the wave function Ψ_n . Since the potential walls are infinitely high, the solution to this equation outside of the box is easy—there is no chance the particle is outside the box so the wave function must be 0. Inside the box, $U = 0$, and Eq. 1-5 can be easily solved. The solution is

$$\Psi_n = A \sin bx \quad (1-6)$$

where A and b are constants. At the ends of the box, Ψ must be zero. This happens when $\sin n\pi = 0$ and n is an integer, so b must be equal to $n\pi/L$. This causes Ψ_n to be 0 when $x = 0$ and $x = L$ for all integral values of n . To evaluate A , we introduce another concept from quantum mechanics, namely that the probability of finding the particle in the interval between x and $x + dx$ is $\Psi^2 dx$. Since the particle must be in the box, the probability of finding the particle in the box is 1, or

$$\int_0^L \Psi_n^2 dx = \int_0^L A^2 \sin^2(n\pi x/L) dx = 1 \quad (1-7)$$

Evaluation of this integral gives $A = \sqrt{2/L}$. Thus the final result for the wave function is

$$\Psi_n = \sqrt{2/L} \sin(n\pi x/L) \quad (1-8)$$

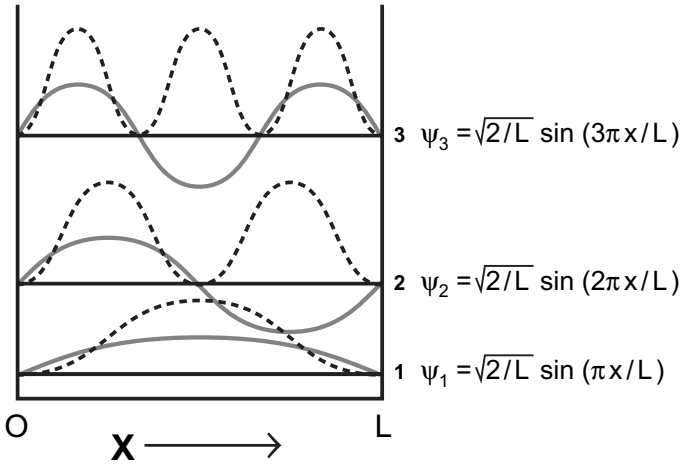


Figure 1-5. Wave functions, Ψ , for the first three energy levels of the particle in a box (Eq. 1-8). The dashed lines show the probability of the finding the particle at a given position x , Ψ^2 .

Obviously n cannot be 0, as this would predict that there is no probability of finding the particle in the box, but n can be any integer. The wave functions for a few values of n are shown in Figure 1-5. Basically Ψ_n is a sine wave, with the “wavelength” decreasing as n increases. (More advanced treatments of quantum mechanics use the notation associated with complex numbers in discussing the wave equation and wave functions, but this is beyond the scope of this text.)

To determine the energy of the particle, all we have to do is put Eq. 1-8 back into Eq. 1-5 and solve for E_n . The result is

$$E_n = (h^2 n^2) / (8mL^2) \quad (1-9)$$

Thus, we see that the energy is quantized, and the energy is characterized by a series of energy levels, as depicted in Figure 1-6. Each energy level, E_n , is associated with a specific wave function, Ψ_n . Notice that the energy levels would be very widely spaced for a very light particle such as an electron, but would be very closely spaced for a macroscopic particle. Similarly, the smaller the box, the more widely spaced the energy levels. For a tennis ball being hit on a tennis court, the ball is sufficiently heavy and the court (box) sufficiently big so that the energy levels would be a continuum for all practical purposes. The uncertainty in the momentum and position of the ball cannot be blamed on quantum mechanics in this case! The particle in a box illustrates how quantum mechanics can be used to calculate the properties of systems and how quantization of energy levels arises. The same calculation can be easily done for a three-dimensional box. In this case, the energy states are the sum

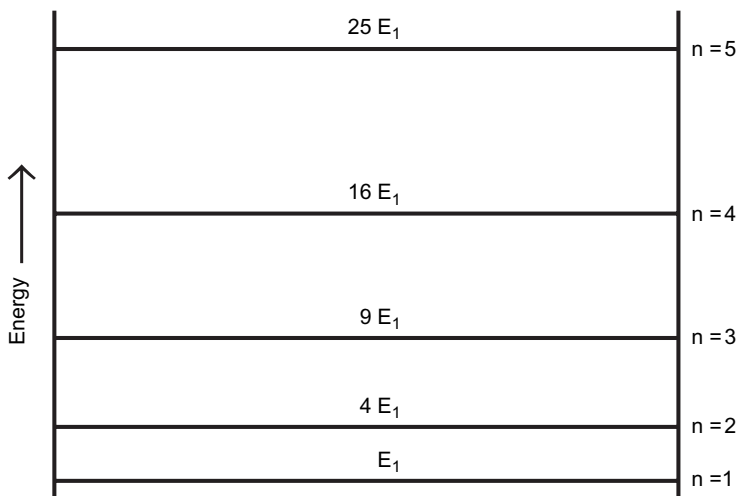


Figure 1-6. Energy levels for a particle in a box (Eq. 1-9). The energy levels are $n^2 E_1$ where E_1 is the energy when $n = 1$.

of three terms identical to Eq. 1-9, but with each of the three terms having a different quantum number.

The quantum mechanical description of matter does not permit determination of the precise position of the particle to be determined, a manifestation of the Heisenberg uncertainty principle. However, the probability of finding the particle within a given segment of the box can be calculated. For example, the probability of finding the particle in the middle of the box, that is, between $L/4$ and $3L/4$ for the lowest energy state is

$$\int_{L/4}^{3L/4} \Psi_1^2 dx = (2/L) \int_{L/4}^{3L/4} \sin^2(\pi x/L) dx$$

Evaluation of this integral gives a probability of 0.82. The probability of finding the particle within the middle part of the box is independent of L , the size of the box, but does depend on the value of the quantum number, n . For the second energy level, $n = 2$, the probability is 0.50. The probability of finding the particle at position x in the box is shown as a dashed line for the first three energy levels in Figure 1-5.

An important result of quantum mechanics is that not only do molecules exist in different discrete energy levels, but the interaction of radiation with molecules causes shifts between these energy levels. If energy or radiation is *absorbed* by a molecule, the molecule can be raised to a higher energy state, whereas if a molecule loses energy, radiation can be *emitted*. For both cases, the change in energy is related to the radiation that is absorbed or emitted by a slight modification of Eq. 1-1, namely the change in energy state of the molecule, ΔE , is

$$\Delta E = h\nu = hc/\lambda \quad (1-10)$$

The change in energy, ΔE , is the difference in energy between specific energy levels of the molecule, for example, $E_2 - E_1$ where 1 and 2 designate different energy levels. It is important to note that since the energy is quantized, the light emitted or absorbed is always a specific single frequency. Equation 1-10 can be applied to the particle in a box for the particle dropping from the $n + 1$ energy level to the n energy level:

$$\Delta E = \frac{h^2}{8mL^2} [(n+1)^2 - n^2] = \frac{h^2}{8mL^2} (2n+1) = hc/\lambda \quad (1-11)$$

If the particle is assumed to be an electron moving in a molecule 20 \AA long and $n = 10$, then $\lambda \sim 600 \text{ nm}$. This wavelength is in the visible region and has been observed for π electrons that are highly delocalized in molecules.

In practice, energy levels are sometimes so closely spaced that the frequencies of light emitted appear to create a continuum of frequencies. This is a shortcoming of the experimental method—in reality the frequencies emitted are discrete entities. The particle in a box is a rather simple application of quantum mechanics, but it illustrates several important points that also are found in more complex calculations for molecular systems. First, the system can be described by a wave function. Second, this wave function permits determination of the probability of important characteristics of the system, such as positions. Finally, the energy of the system can be calculated and is found to be quantized. Moreover, the energy can only be absorbed or emitted in quantized packages characterized by specific frequencies. Quantum mechanical calculations also tell us what conditions are necessary for energy to be emitted or absorbed by a molecule. These calculations tell us *whether* radiation will be emitted or absorbed and what quantized packets of energy are available. We will only utilize the results of these calculations and will not be concerned with the details of the interactions between light and molecules other than the above concepts.

PROPERTIES OF WAVES

It is useful to consider several additional aspects of light waves in order to understand better some of the experimental methods that will be discussed later. Thus far we have considered light to be a periodic electromagnetic wave in space that could be characterized, for example, by a sine function:

$$I = I_0 \sin(2\pi x/\lambda) \quad (1-12)$$

Here I is the magnetic or electric field, I_0 is the maximum value of the electric or magnetic field, x is the distance along the x axis and λ is the wavelength.

A light wave can also be periodic in time, as illustrated in Figure 1-7. In this case:

$$I = I_0 \sin 2\pi\nu t = I_0 \sin \omega t \quad (1-13)$$

Now, I is the light intensity, I_0 is the maximum light intensity, ν is the frequency in s^{-1} , as defined in Figure 1-7, ω is the frequency in radians ($\omega = 2\pi\nu$), and t is the time. The velocity of the propagating wave is $\lambda\nu$, which in the case of electromagnetic radiation is the speed of light, that is, $\lambda\nu = c$. If light of the same frequency and maximum amplitude from two sources is combined, the two sine functions will be added. If the two light waves start with zero intensity at the same time ($t = 0$), the two waves add and the intensity is doubled. This is called *constructive interference*. If the two waves are combined with one of the waves starting at zero intensity and proceeding to positive values of the sine function, whereas the other begins at zero intensity and proceeds to negative values, the two intensities cancel each other out. This is called *destructive interference*. Obviously it is possible to have cases in between these two extremes. In such cases, a phase difference is said to exist between the two waves. Mathematically this can be represented as

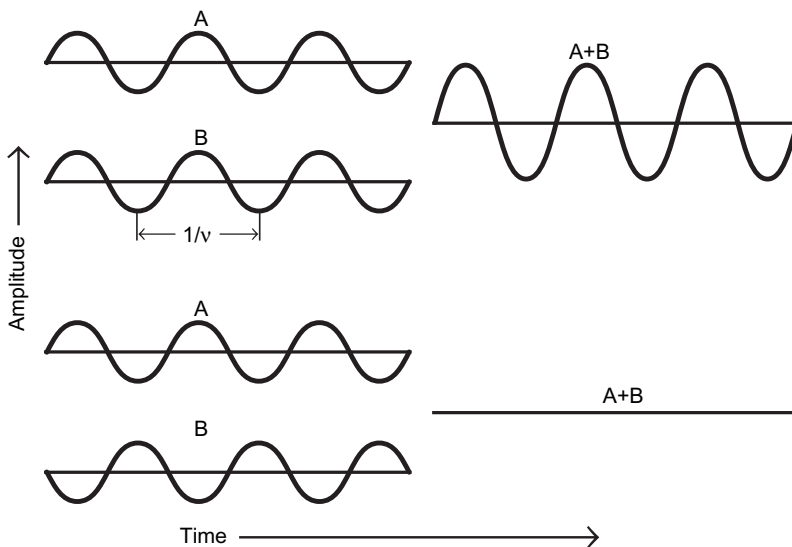


Figure 1-7. Examples of constructive and destructive interference. Constructive interference: when the upper two wave forms of equal amplitude and a phase angle of 0° (or integral multiples of 2π) are added (*left*), a sine wave with twice the amplitude and the same frequency results (*right*). Destructive interference: when the lower two wave forms are added (*left*), the amplitudes of the two waves cancel (*right*). The phase angle in this case is 90° (or odd integral multiples of $\pi/2$).

$$I = I_0 \sin(\omega t + \delta) \quad (1-14)$$

where δ is called a phase angle and can be either positive or negative. When many different waves of the same frequency are combined, the intensity can always be described by such a relationship. These phenomena are shown schematically in Figure 1-7.

A standard way of carrying out spectroscopy is to apply continuous radiation, and then look at the intensity of the radiation after it has passed through the sample of interest. The intensity is then determined as a function of the frequency of the radiation, and the result is the absorption spectrum of the sample. The color of a material is determined by the wavelength of the light absorbed. For example, if white light shines on blood, blue/green light is absorbed so that the transmitted light is red. Several examples of absorption spectra are shown in Figure 1-8. We will consider why and how much the sample absorbs light a bit later, but you are undoubtedly already familiar with the concept of an absorption spectrum.

The use of continuous radiation is a useful way to carry out an experiment, but there is an interesting mathematical relationship that permits a different approach to the problem. This mathematical operation is the *Fourier transform*. The principle of a Fourier transform is that if the frequency dependence of the intensity, $I(\nu)$, can be determined, it can be transformed into a new function, $F(t)$, that is a function of the time, t . Conversely, $F(t)$ can also be converted to $I(\nu)$. Both of these functions contain the same information. Why then are these transformations advantageous? It can be quite time consuming to determine $I(\nu)$, but a short pulse of radiation can be applied very quickly. Basically what this transformation means is that looking at the response of the system to application of a pulse of radiation, such as shown as in Figure 1-9, is equivalent to looking at the response of the system to sine wave radiation at many different frequencies. In other words, a square wave is mathematically equivalent to adding up many sine waves of different frequency, and vice versa. This is shown schematically in Figure 1-9 where the addition of sine waves with four different frequencies produces a periodic “square” wave. The larger the number of sine waves added, the more “square” the wave becomes. In mathematical terms, a square wave can be represented as an infinite series of sine functions, a Fourier series.

The mathematical equivalence of timed pulses and continuous waves of many different frequencies has profound consequences in determining the spectroscopic properties of materials. In many cases, the use of pulses permits thousands of experiments to be done in a very short time. The results of these experiments can then be averaged, producing a far superior frequency spectrum in a much shorter time than could be determined by continuous wave methods. In later chapters, we will be dealing both with continuous wave spectroscopy and Fourier transform spectroscopy. It is important to remember that

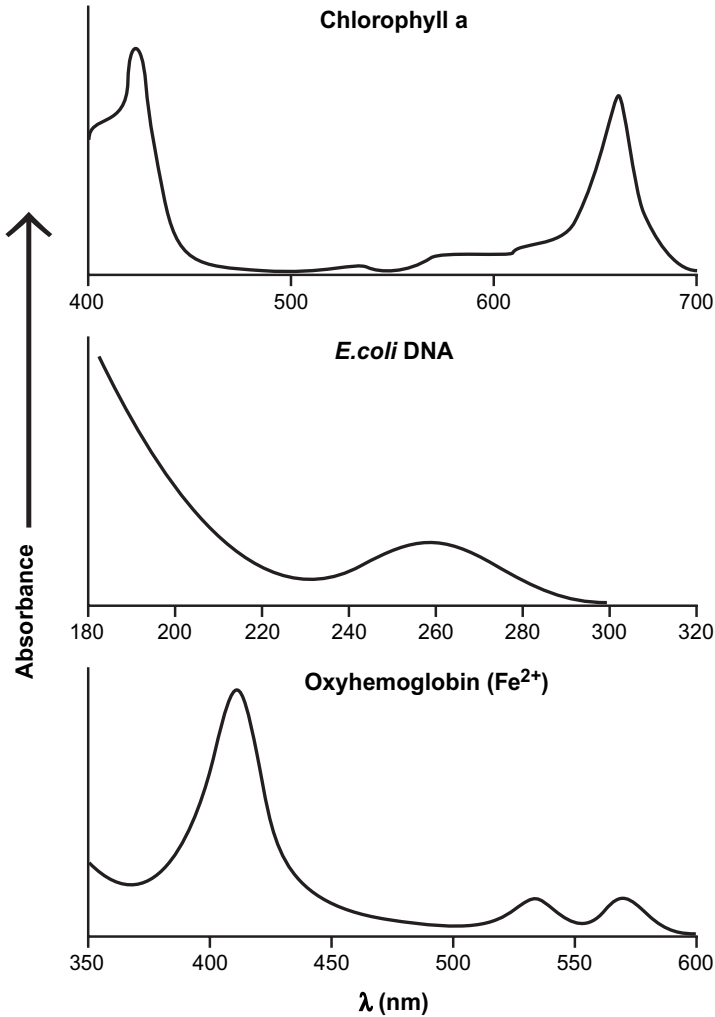


Figure 1-8. Absorption of light by biological molecules. The absorbance scale is arbitrary and the wavelength, λ , is in nanometers. Chlorophyll *a* solutions absorb blue and red light and are green in color. DNA solutions absorb light in the ultraviolet and are colorless. Oxyhemoglobin solutions absorb blue light and are red in color.

both methods give identical results. The method of choice is that one that produces the best data in the shortest time, and in some cases at the lowest cost.

With this brief introduction to the underlying theoretical principles of spectroscopy, we are ready to proceed with consideration of specific types of spectroscopy and their application to biological systems.

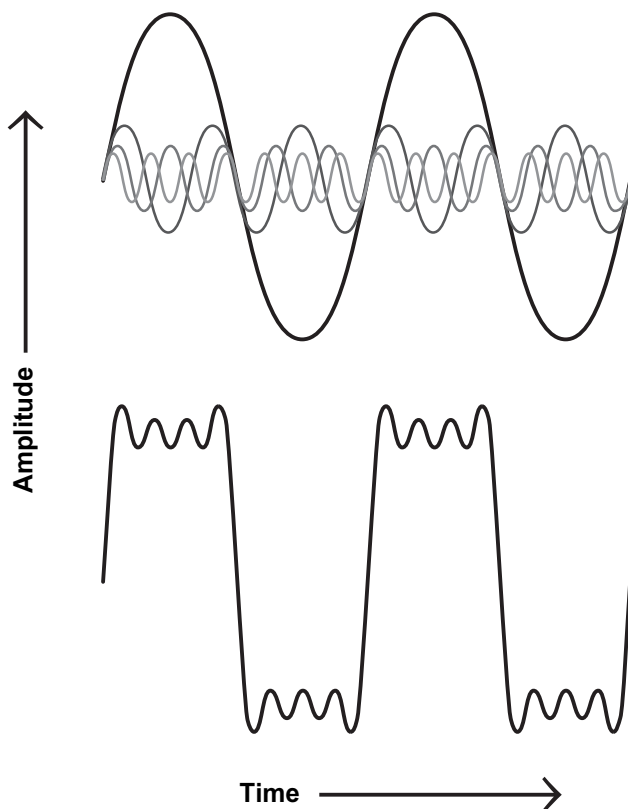


Figure 1-9. The upper part of the figure shows sine waves of four different frequencies, and the lower part of the figure is the sum of the sine waves, which approximates a square wave pulse of radiation. When sine waves of many more frequencies are included, the time dependence becomes a pulsed square wave. This figure illustrates that the superposition of multiple sine waves is equivalent to a square wave pulse and vice versa. This equivalency is the essence of Fourier transform methods. Copyright by Professor T. G. Oas, Duke University. Reproduced with permission.

REFERENCES

The topics in this chapter are discussed in considerably more depth in a number of physical chemistry textbooks, such as those cited below.

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2. R. J. Silbey, R. A. Alberty, and M. G. Bawendi, *Physical Chemistry*, 4th edition, John Wiley & Sons, New York, 2004.

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PROBLEMS

- 1.1. The energies required to break the C–C bond in ethane, the “triple bond” in CO, and a hydrogen bond are about 88, 257, and 4 kcal/mol. What wavelengths of radiation are required to break these bonds?
- 1.2. Calculate the energy and momentum of a photon with the following wavelengths: 150 pm (x ray), 250 nm (ultraviolet), 500 nm (visible), and 1 cm (microwave).
- 1.3. The maximum kinetic energy of electrons emitted from Na at different wavelengths was measured with the following results.

λ (Å)	Max Kinetic Energy (electron volts)
4500	0.40
4000	0.76
3500	1.20
3000	1.79

Calculate Planck’s constant and the value of the work function from these data. (1 electron volt = 1.602×10^{-19} J)

- 1.4. Calculate the de Broglie wavelength for the following cases:
 - a. An electron in an electron microscope accelerated with a potential of 100 kvolts.
 - b. A He atom moving at a speed of 1000 m/s.
 - c. A bullet weighing 1 gram moving at a speed of 100 m/s.

Assume the uncertainty in the speed is 10%, and calculate the uncertainty in the position for each of the three cases.

- 1.5. The particle in a box is a useful model for electrons that can move relatively freely within a bonding system such as π electrons. Assume an electron is moving in a “box” that is 50 \AA long, that is, a potential well with infinitely high walls at the boundaries.
 - a. Calculate the energy levels for $n = 1, 2,$ and 3 .
 - b. What is the wavelength of light emitted when the electron moves from the energy level with $n = 2$ to the energy level with $n = 1$?

- c. What is the probability of finding the electron between 12.5 and 37.5 Å for $n = 1$.
- 1.6. Sketch the graph of I versus t for sine wave radiation that obeys the relationship $I = I_0 \sin(\omega t + \delta)$ for $\delta = 0, \pi/4, \pi/2$, and π .

Plot the sum of the sine waves when the sine wave for $\delta = 0$ is added to that for $\delta = 0$ or $\pi/4$, or $\pi/2$, or π . This exercise should provide you with a good understanding of constructive and destructive interference.

Do your results depend on the value of ω ? Briefly discuss what happens when waves of different frequency are added together.

