Infrared Spectroscopy Course for Entomologists

Mario González-Jiménez

v 2.0 (29-02-2024)

CHAPTER 1

PHYSICAL PRINCIPLES OF INFRARED SPECTROSCOPY

§ 1. *The motion of free atoms*

We can think of the particles that make up matter, the atoms, as tiny billiard balls. These particles convert the energy they receive in the form of heat into motion, in fact, temperature is a measure of the amount of motion the particles of a material have. These motions can be in two forms: displacement and rotation. One of these tiny balls will move and rotate indefinitely and at the same speed until it interacts with another particle. If they collide, for example, the principle of conservation of energy[1] says that the energy lost by one particle will be gained by the other. And so the energy of the whole, if it is isolated from the outside,[2] will remain constant over time.

§ 2. *Electronegativity: atoms and electricity*

Atoms are made up of protons, neutrons and electrons. Protons and neutrons provide the mass of an atom and are found in its nucleus. Neutrons have no electric charge, but protons have a positive electric charge. Around the nucleus orbit electrons, which have the same amount of electrical charge as a proton, but the charge of an electron is negative. Electric charges work in the same way as the poles of a magnet. Equal charges (positive and positive or negative and negative) repel each other, and different charges (negative and positive) attract each other.

---

[1] The principle of conservation of energy says that energy is neither created nor destroyed, it is only transformed.

[2] Like in a thermos flask

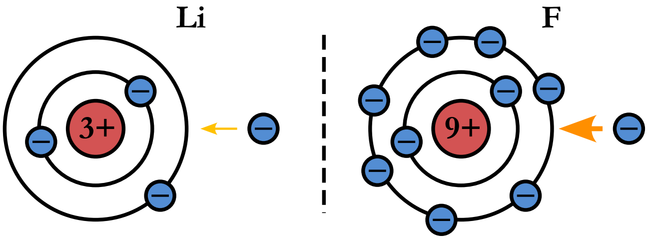
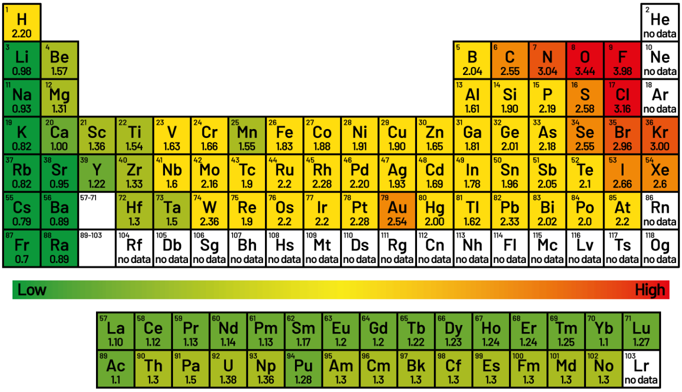


Figure 1.1: Diagram of the attraction of a free electron to the nuclei of lithium and fluorine. This tendency of atoms to attract electrons that are not their own is known as electronegativity.

An electron has exactly the same amount of negative charge as a positively charged proton. Therefore, atoms, which have the same number of protons as electrons, have no *net* electric charge. I have emphasised the word net because in reality atoms are not as neutral as one would expect. The reason is because while all their positive electric charge is concentrated in the nucleus, all the negative electric charge is distributed in layers surrounding the nucleus. This is because the size of the atom means that only a certain number of electrons fit into each shell. Two electrons go into the first shell, eight into the second, eight into the third, but 18 into the fourth, and I could go on until the end. Due to this layered distribution of the electrons, the positive charge of the nucleus is compensated or shielded with different efficiencies.

Take, for example, the lithium atom (figure 1.1). Lithium has three protons in its nucleus and three electrons in its shell, two in the first shell and one in the second shell. Then its three positive charges in the nucleus are shielded by two electron shells. If we now look at fluorine, which has nine protons and nine electrons, we see that its nucleus has nine positive charges and the nine negative charges of its electrons are distributed in two shells as well, with two electrons in the first shell and seven in the second. It is



1 H 2.20																	2 He no data
3 Li 0.98	4 Be 1.57											5 B 2.04	6 C 2.55	7 N 3.04	8 O 3.44	9 F 3.98	10 Ne no data
11 Na 0.93	12 Mg 1.31											13 Al 1.61	14 Si 1.90	15 P 2.19	16 S 2.58	17 Cl 3.16	18 Ar no data
19 K 0.82	20 Ca 1.00	21 Sc 1.36	22 Ti 1.54	23 V 1.63	24 Cr 1.66	25 Mn 1.55	26 Fe 1.83	27 Co 1.88	28 Ni 1.91	29 Cu 1.90	30 Zn 1.65	31 Ga 1.81	32 Ge 2.01	33 As 2.18	34 Se 2.55	35 Br 2.96	36 Kr 3.00
37 Rb 0.82	38 Sr 0.95	39 Y 1.22	40 Zr 1.33	41 Nb 1.6	42 Mo 2.16	43 Tc 1.9	44 Ru 2.2	45 Rh 2.28	46 Pd 2.20	47 Ag 1.93	48 Cd 1.69	49 In 1.78	50 Sn 1.96	51 Sb 2.05	52 Te 2.1	53 I 2.66	54 Xe 2.6
55 Cs 0.79	56 Ba 0.89	57-71	72 Hf 1.3	73 Ta 1.5	74 W 2.36	75 Re 1.9	76 Os 2.2	77 Ir 2.2	78 Pt 2.28	79 Au 2.54	80 Hg 2.00	81 Tl 1.62	82 Pb 2.33	83 Bi 2.02	84 Po 2.0	85 At 2.2	86 Rn no data
87 Fr 0.7	88 Ra 0.89	89-103	104 Rf no data	105 Db no data	106 Sg no data	107 Bh no data	108 Hs no data	109 Mt no data	110 Ds no data	111 Rg no data	112 Cn no data	113 Nh no data	114 Fl no data	115 Mc no data	116 Lv no data	117 Ts no data	118 Og no data

57 La 1.10	58 Ce 1.12	59 Pr 1.13	60 Nd 1.14	61 Pm 1.13	62 Sm 1.17	63 Eu 1.2	64 Gd 1.2	65 Tb 1.22	66 Dy 1.23	67 Ho 1.24	68 Er 1.24	69 Tm 1.25	70 Yb 1.1	71 Lu 1.27
89 Ac 1.1	90 Th 1.3	91 Pa 1.5	92 U 1.38	93 Np 1.36	94 Pu 1.28	95 Am 1.3	96 Cm 1.3	97 Bk 1.3	98 Cf 1.3	99 Es 1.3	100 Fm 1.3	101 Md 1.3	102 No 1.3	103 Lr no data

Figure 1.2: Periodic table of the elements with their electronegativity measured empirically on a scale called the Pauling scale. Note how the electronegativity increases as we move up and to the right. Source: Anne Helmenstine - sciencenotes.org

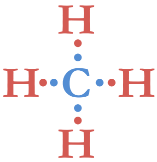
evident that by having much more positive charge counterbalanced by the same number of negative shells, although the second shell is denser than in lithium, fluorine is much less efficient at shielding its positive charge. So if an electron passes near a fluorine atom, it will be much more attracted to it than if it passes near a lithium atom. This ability of atoms to attract electrons due to this effect is called **electronegativity** and is probably, as we will see later, the most important property of an atom in chemistry because it regulates how atoms relate to each other. In figure 1.2 you can see the electronegativity of each atom in the periodic table. As we move down the table, the atom has a new layer, the electronegativity decreases. Then, as all the atoms in each row have the same number of layers, as we move to the right and thus increase the positive charge of the nucleus, the electronegativity increases.

§ 3. *Molecules: Atoms bonded together*

There are very few elements that occur naturally as single atoms. At normal temperatures, the noble gases and little else. Normally, atoms are more comfortable bonded to other atoms to form molecules. The bonding between two or more atoms occurs through a chemical bond. There are three types of chemical bond. To understand them better, it is necessary to know how atoms are arranged to form molecules. It all depends on the valence shell. This is the outermost electron shell of an atom. Atoms that have a full valence shell are much more stable than other atoms. If we look at the periodic table, the atoms that naturally have a full valence shell are helium, neon, krypton... i.e. the noble gases. Their electronic configuration is so stable that they do not react with other elements because they do not need to. The rest of the elements try to get a full valence layer in any way they can. Those that have a valence shell with one or two electrons tend to lose them in order to remove the last shell and turn the innermost shell into a full valence shell. They do this regardless of the fact that they are left without some electrons and are therefore left with a net positive charge, becoming cations. Something similar happens with elements that are missing one or two electrons to complete their valence shell. They tend to steal those missing electrons from other elements to become an anion with a full shell. When anions and cations are present in a medium, their positive and negative charges cause them to attract each other to form huge networks of atoms that together have a neutral charge, called salts. This type of bonding is called **ionic bonding**.

Sometimes, if an element with few valence electrons cannot give them up to any other element, its atoms join together and begin to share their valence electrons with each other, creating a cloud of electrons that move freely around the nuclei that hold them together. This type of bonding is a **metallic bond**. The valence electrons are not associated with any specific atom, but move freely through the structure of the metal.

These two types of bonding generate aggregates of an immense number of atoms. There is a third type of bond that allows the formation of molecules as small as a diatomic molecule or as large as a protein or DNA. It is the **covalent bonding**. In covalent bonding, the bond between two atoms occurs when two atoms share one, two or three electrons with each other because in doing so they fill their valence shell with the remaining electrons and thus achieve the desired stability. For example, methane is produced when a carbon shares its four valence electrons with four hydrogens, which in return share their one electron with the carbon. In this way, the hydrogens get two electrons in their valence shell and fill it up, and the carbon gets eight electrons in its valence shell and fills it up as well. As the bond is formed with only one pair of shared electrons, it is called a **single bond**.



If two elements need to share two electrons with each other to complete their valence shell, they will do so without hesitation. This is a **double bond** and is about twice as strong as a single bond.

Elements can bond by sharing up to three electrons with each other to form a **triple bond**. A triple bond is about three times stronger than a single bond. This makes this kind of bond very resistant to breaking. An example of this type of bond is nitrogen, the most inert diatomic gas.[3]

---

[3] Fortunately, because it forms 79% of our atmosphere!

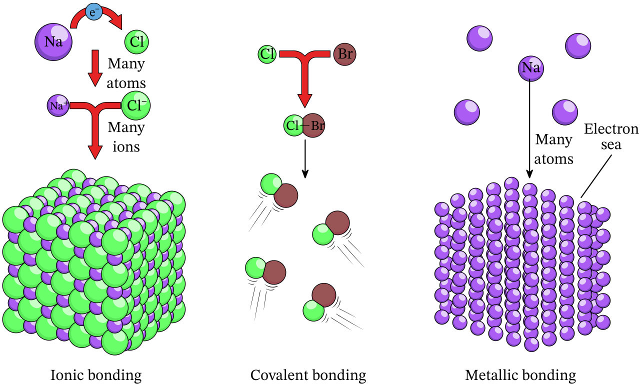


Figure 1.3: The three types of chemical bonds.

§ 4. *Vibration: Motion of two atoms joined together.*

Going back to the analogy with billiard balls, a bond does not stop billiard balls from moving. The bond is elastic, which allows them some freedom of movement, and essentially behaves as if the two balls were held together by a spring or a rubber band, so that any attempt to separate them becomes a vibration. Furthermore, the bonded atoms continue to rotate and displace as a single body.

Let's take a closer look at this process. Two atoms are bonded together. The position in which the two would be at ease if they were not moving is called the equilibrium position. Imagine that one of them is fixed and cannot move. Meanwhile, the energy of its motion, or kinetic energy, causes the other atom to move away from the first one. The further apart they are, the more the bond will resist stretching, which means that the moving atom will have to work harder and harder to continue on its way. The force against it can therefore be expressed mathematically as follows:

$$F = -kx \tag{1.1}$$

This expression is called Hooke's law and in it, F is the force opposing the motion of the atom (hence its negative sign), x is the distance to the equilibrium position and k is how hard the bond is, the harder it is the greater the force opposing the motion.

In the process of moving away from the fixed atom, the particle loses its kinetic energy, which is stored by the bond as elastic potential energy. When it reaches the end of its path and stops, the bond pulls the particle back, releasing the potential energy as kinetic energy. The particle accelerates with the force of Hooke's equation, now in favour, until it reaches the equilibrium position and then the bond begins to resist compressing and the process begins again, starting a constant vibrating motion.

It is not very difficult to find an equation that allows us to predict the position of the atom at any given moment. Newton's second law says that the force needed to change the velocity of a body (accelerate or decelerate it) is proportional to its mass m, which is the same:

$$F = ma \tag{1.2}$$

Since we know the force that keeps the body in motion (equation 1.1), we can solve the equation

$$ma = -kx \tag{1.3}$$

Where is the time? Acceleration is the change of velocity with time and velocity is the change of position with time, so acceleration is the change of the change of position with time, that is, the second derivative of position with respect to time:

$$a = \frac{d^2x}{dx^2} \tag{1.4}$$

Knowing this, the equation of motion of the oscillating particle (equation 1.3) looks like this:

$$m\frac{d^2x}{dx^2} = -kx \tag{1.5}$$

This is a differential equation, which is an equation involving a derivative. Let us forget for a moment about m and k, imagining that, for example, $m = 1$ kg and $k = 1$ N/m. The equation 1.5 would look like this:

$$\frac{d^2x}{dx^2} = -x \tag{1.6}$$

To solve this equation, then, you have to do the same thing you do with all differential equations. Read it, see what it means, and look for the mathematical function that fulfils it. In this case we must ask ourselves: "*which function, when it is derived twice, is equal to itself but in negative?*" The answer is the cosine!

$$x = \cos t \tag{1.7}$$

Verification:

$$\frac{dx}{dt} = \frac{d\cos t}{dt} = -\sin t$$
$$\frac{d^2x}{dt^2} = \frac{d(-\sin t)}{dt} = -\cos t = -x$$

If we now recover m and k (and pass m to the other side of the equation), the question hardly changes: "what function when twice derived is equal to itself in negative and multiplied by k/m?"

$$\frac{d^2x}{dx^2} = -\frac{k}{m}x \tag{1.8}$$

The answer is still the cosine, but for a constant (k/m) to appear in the derivative, the cosine must have something in its interior. Let's call that something, for the time being, ω:

$$x = \cos\omega t \tag{1.9}$$

Its two derivatives are:

$$\begin{aligned}\frac{dx}{dt} &= \frac{d\cos\omega t}{dt} = -\omega\sin\omega t\\ \frac{d^2x}{dt^2} &= \frac{d(-\omega\sin\omega t)}{dt} = -\omega^2\cos\omega t\end{aligned}$$

And now we can see to find out what ω is. The second derivative of $\cos\omega t$ will only be equal to $-k/mx$ if:

$$\omega^2 = \frac{k}{m}$$

or what is the same:

$$\omega = \sqrt{\frac{k}{m}}.$$

So the solution is:

$$x = \cos\sqrt{\frac{k}{m}}t. \tag{1.10}$$

This solution makes perfect sense. The cosine is an oscillatory function that is ideal for describing the oscillation of a particle. It is also a function that passes through the same place every time its argument is a multiple of 2π. This tells us that the time needed to complete an oscillation will be:

$$\sqrt{\frac{k}{m}}t = 2\pi \Rightarrow$$

$$\Rightarrow t = 2\pi\sqrt{\frac{m}{k}}. \tag{1.11}$$

The **frequency** (f) is the number of oscillations that take place in a unit of time. It is the inverse function of the duration of an oscillation:

$$f = \frac{1}{t} \tag{1.12}$$

So in our system the frequency will be given by:

$$f = \frac{1}{t} = \frac{1}{2\pi\sqrt{\frac{m}{k}}}$$

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} \tag{1.13}$$

But this is for the oscillation of one atom when the other is fixed. But let us remember that our bond consisted of two atoms, sometimes of different mass, moving back and forth. We can use the formula we have deduced above to describe the motion of these two atoms. For this, we only need to use the reduced mass. When two objects, such as atoms or molecules, are bound together by a force, they move in a coordinated way around their common center of mass. The reduced mass is a way to simplify the problem of describing their motion by considering the two objects as a single object with an effective mass placed in the center of mass. This effective mass is the reduced mass and can be calculated, for two bodies with masses m_1 y m_2 with the equation:

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \tag{1.14}$$

So the frequency of vibration of a bond is given by:

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \tag{1.15}$$

From this equation we can deduce a couple of things:

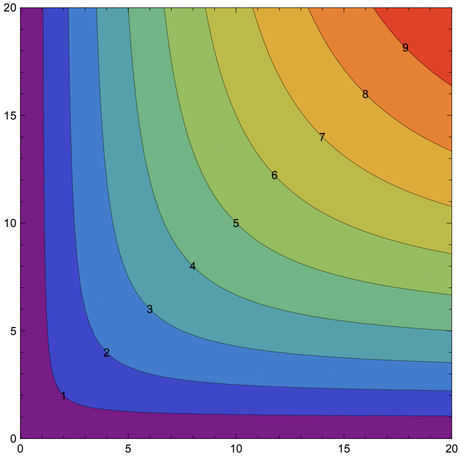


Figure 1.4: Reduced mass μ of a diatomic molecule as a function of the masses of its two atoms.

1. If the two atoms are equal, the heavier they are, the slower they will vibrate because their inertia[4] is greater. However, if one atom is very heavy and the other very light, the light one cannot pull the heavy one and what happens is that the heavy one barely moves and it is the light one that suffers all the bonding force vibrating with a very high frequency.[5].

2. The greater the strength of the bond k, the faster the molecule will vibrate. The table 4. shows typical force constants for different bonds. Note that for molecules with the same atoms, the triple bond constants are higher than the double bond constants and the double bond constants are higher than the single bond constants. Therefore,

---

[4] The resistance to change speed of a body depends on its mass.

[5] As occurs in all the bonds in which hydrogen participates (see page 51)

Bond	k / N/m
H–H	570
H–F	970
H–Cl	480
H–Br	410
H–I	320
C=O	1020
N=O	1530
C–C	540*
C=C	1000*
C≡C	1500*
N≡N	2287
O=O	1133

Table 1.1: Strength constants k of the most common bonds. *Approximate constant, depends on the surrounding atoms.

> the frequencies of the triple bonds are higher than those of the double bonds and the double bonds are higher than those of the single bonds (see page 56).

Note that the frequency of vibration is totally independent of the amplitude of vibration. The same is true for springs, the frequency will always be the same regardless of the force pulled on them. This property can be used to use harmonic movements as clocks. Although the frequency does not depend on the amplitude, the energy of a vibrating bond does depend on the amplitude. It is necessary to apply a force to make it vibrate in a wider way. When a force causes an object to change its motion, it is said to be doing work W. The amount of work done is equal to the force times the distance moved.

To quantify it perfectly, we add up the forces F_x that have been realized in all the units of distance travelled, considering that they are infinitely small dx. This is to make an integral between the initial atom spacing x_i and the final x_f:

$$W = \int_{x_i}^{x_f} F_x dx \tag{1.16}$$

We introduce the force equation (equation 1.1)

$$W = \int_{x_i}^{x_f} -kxdx = \left[-\frac{1}{2}kx^2\right]_{x_i}^{x_f} = -\left[\frac{1}{2}kx_f^2 - \frac{1}{2}kx_i^2\right] =$$

The two subtraction terms are the potential energy of the bond at the end and at the beginning:

$$= -[E_f - E_i] = -\Delta E$$

Then the potential energy stored in a bond depends quadratically on the deviation from the equilibrium position:

$$E = \frac{1}{2}kx^2 \tag{1.17}$$

Knowing this, we can draw the curve that indicates the potential energy that a bond will have as a function of the spacing of its atoms (figure 1.5).

§ 5. *Electromagnetic radiation*

When an electrically charged particle is accelerated, it alters the space around it in the same way that when a stone falls into a lake it alters the surface of the water: by generating a wave. The wave generated is called an electromagnetic wave because what oscillates or vibrates is nothing material but electric and magnetic force fields. A force field is not something material, it is only a property of a particular point in space that means that if a particle sensitive to that force is in it, it will suffer a force. The simplest example is the magnetic field generated by a magnet. It is nothing material, but its forces can be easily visualized by sprinkling iron filings around it (figure 1.6).

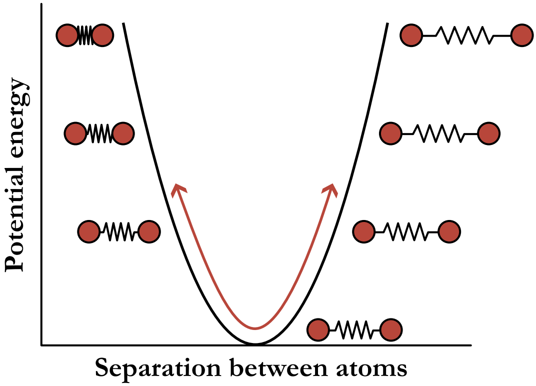


Figure 1.5: Potential energy curve for a classical harmonic oscillator. The more the bond is shortened and lengthened, the more potential energy the vibration reaches.

One advantage of the fact that what oscillates is not material is that it does not attenuate with time or distance as evidenced by the fact that right now the Earth is receiving radiation from the edge of the universe. Also that it travels very fast. An electromagnetic wave travels at 299,792.458 kilometres per second. This speed is known as the speed of light and is represented by the letter c.

Since we know its speed, we can know the relationship between all the properties of the wave. Since a wave is periodic, it has four properties:

1. **Wavelength** (λ): The distance between two points in the wave of equal characteristics. (figure 1.7).

2. **Period** (T): The time it takes for the next point of equal characteristics to appear. Since it is the time it takes to travel the wavelength,

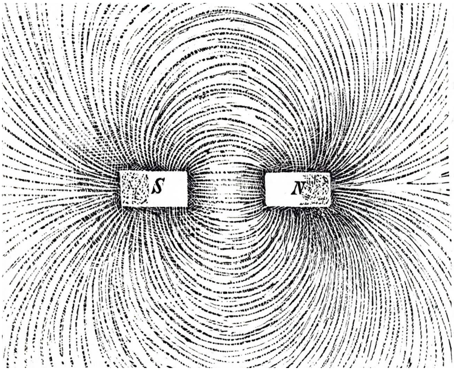


Figure 1.6: Visualisation of the magnetic field around the poles of a magnet.

and we know the speed it is easy to relate λ y T:

$$T = \frac{\lambda}{c} \tag{1.18}$$

3. **Frequency** (ν): The number of oscillations that the wave makes during a unit of time.

$$\nu = \frac{1}{T} \tag{1.19}$$

From the equation 1.18 we can derive the relationship between frequency and wavelength:

$$\lambda = c\nu \tag{1.20}$$

4. **Wavenumber** ($\tilde{\nu}$): It is the number of oscillations the wave makes in a unit of distance.

$$\tilde{\nu} = \frac{1}{\lambda} \tag{1.21}$$

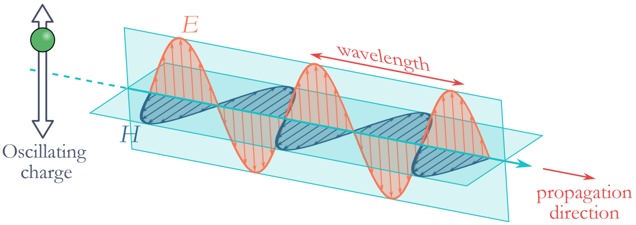


Figure 1.7: The oscillation of a charge generates an electromagnetic wave, a self-propagating perturbation of an electric field E and a magnetic field H. The distance between two equivalent points of the wave is the wavelength λ.

Any of these four properties can be used to explain the characteristics of a wave.

§ *6. Interaction of molecules with infrared radiation*

As the electromagnetic wave is a disturbance in the electric field, this means that only particles that have electric charge can feel it. That is, if a wave reaches an uncharged particle nothing will happen, but if a charged particle crosses the path of the electromagnetic radiation it will experience the energy it carries in the form of a force that will move it.

This also happens with molecules. If the two atoms of a molecule are equal, the electric charges of their electrons (negative) and nuclei (positive) will be equally distributed between the two and therefore the disturbance of electromagnetic radiation will not affect them. However, if the two atoms in a bond are different, the more electronegative atom will attract the electrons of the two, charging itself with a negative charge and leaving the other atom with a positive charge. This bond constitutes an electric dipole. The intensity of a dipole is called the dipole moment. The greater the difference in electronegativities between the two atoms in the bond, the greater their dipole moment. When an electromagnetic wave reaches a dipole molecule, if its frequency coincides exactly with the frequency at which the molecule is vibrating, it transfers its energy to the molecule and the molecule increases the amplitude of its vibration. Molecules usually vibrate with a frequency that is in the range of infrared radiation. The higher the dipole moment of a molecule, the more it interacts with the infrared radiation and the more it retains its energy, absorbing the radiation.

Climate change is due to molecules absorbing infrared radiation emitted by the ground after heating by UV-visible radiation from the sun (greenhouse effect). Of the most common molecules contained in the atmosphere, most have no dipole moment because they are symmetric or monoatomic and do not contribute to the greenhouse effect: Nitrogen (N≡N, 78%), oxygen (O=O, 21%), argon (Ar, 0.93%), neon (Ne, 0,002%), helium (He, 0,0005%), krypton (Kr, 0,0001%) and hydrogen (H–H, 0,00005%). However, there are three molecules that do contribute to climate change: carbon dioxide (C=O=C, 0,033%), methane (CH_4, 0,00015%) and water vapour (H–O–H, the proportion of which depends on the weather).

§ 7. *Vibration of polyatomic molecules*

Since the section 1. (page 3), we have been talking about vibrations as a consequence of the thermal motion of two joined atoms. So far we have been talking about diatomic molecules whose vibration is very simple. When a molecule has several atoms then there can be many more ways to vibrate. Each of these ways of vibrating is called a vibrational mode and all vibrational modes can be classified into two categories (figure 1.8):

1. Stretching vibrations: When the motion of the atoms occurs on the same axis as that of the bond and therefore the vibration only changes its length. Depending on the timing between the two atoms involved, stretching vibrations can be further divided into:

    (a) Symmetrical stretching: When the two atoms move towards and away from the central atom at the same time.

    (b) Asymmetric stretching: When one of the atoms approaches the central atom while the other, synchronously, moves away from it.

2. Bending vibrations: When the motion of the two atoms attached to the central atom is such that the angle between them changes, but their bond distance remains constant. This type of vibration requires less energy than stretching and therefore appears at lower frequencies. These vibrational modes can occur in the same plane or changing planes:

    (a) Rocking: When, in the plane, the two atoms move to the same direction causing them to oscillate synchronously with the central atom.

    (b) Scissoring: When, in the plane, the two atoms move in opposite directions causing them to oscillate towards and away from each other continuously.

    (c) Wagging: The two atoms move up and down the plane of the central atom at the same time.

    (d) Twisting: One atom rises above the plane of the central atom while the other one descends, and harmonically they return the other way round.

Vibrational modes also have to be polar in order to interact with infrared radiation. For example, symmetric stretch vibrational modes in symmetric, linear molecules, such as carbon dioxide (O=C=O), are not active.

Stretching vibrations tend to have higher frequencies than bending vibrations because stretching a bond typically requires more energy than twisting it. Also, symmetric stretching is an easier deformation than asymmetric stretching because it doesn't change the dipole moment of the molecule, so it always occurs at lower frequencies than asymmetric stretching.

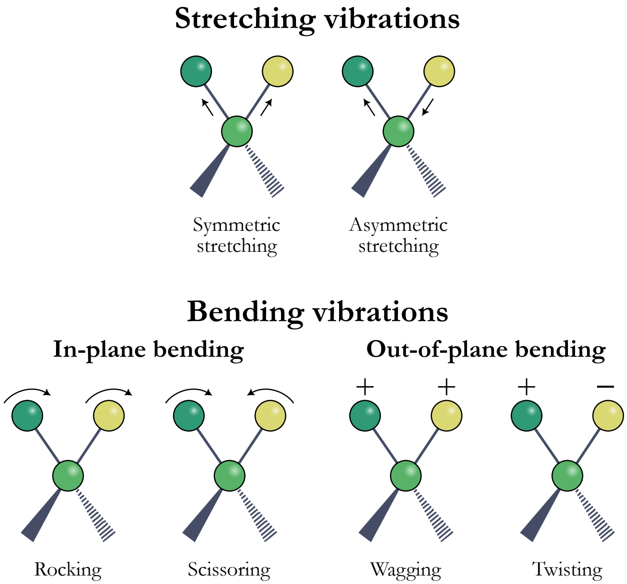


Figure 1.8: Types of vibrations in polyatomic molecules.

In the case of bending, in-plane vibrations tend to have higher frequencies than out-of-plane vibrations. Scissoring vibrations tend to have higher frequencies than rocking vibrations. This is because scissoring involves a greater change in bond angles and potentially higher restoring forces than the side-to-side motion of rocking. For out-of-plane vibrations, wagging vibrations tend to have slightly higher frequencies than twisting vibrations. This is because waggling motions generally involve less rotational inertia than twisting motions, resulting in a higher frequency for a given set of bond strengths and atomic masses.

§ *8. Quantisation*

Electromagnetic radiation carries its energy in small packets called photons. Each photon carries an amount of energy that depends on the frequency of its wave:

$$E = h\nu \tag{1.22}$$

where h is Planck's constant, which is $6.626 \cdot 10^{-34}$ J s.

The fact that light is actually transported in specific amounts of energy is called quantisation and, as the tiny value of Planck's constant shows, it is an effect that is only noticeable for very small amounts of energy. Precisely, the energy involved in the vibration of a molecule is very small, and because it is in the range of energies in which quantisation occurs, the motions of the molecule are quantized. This means that a given bond can only vibrate at certain amplitudes, which are determined by this quantum effect. The energies of each of the allowed amplitudes are called levels, and a photon can only be absorbed by the molecule when its energy coincides with the difference between these two energy levels.

The energy of the levels is given by the equation:

$$E = \left(n + \frac{1}{2}\right) \frac{h}{2\pi} \sqrt{\frac{k}{\mu}} \tag{1.23}$$

or:

$$E = \left(n + \frac{1}{2}\right) hf \tag{1.24}$$

where f is the vibrational frequency of the bond, which we calculated in the equation 1.15 and n is the vibrational level number.

We have said that a molecule only absorbs a photon when its energy is equal to the difference of the two levels, so it is easy to calculate the energy and frequency of the photon that is absorbed:

$$\Delta E_{n \rightarrow n+1} = \left((n+1) + \frac{1}{2}\right) hf - \left(n + \frac{1}{2}\right) hf =$$

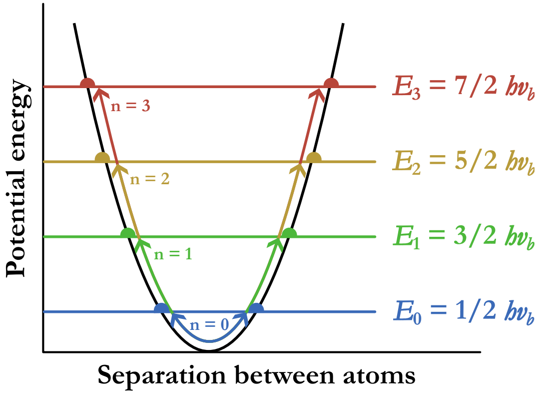


Figure 1.9: The potential energy curve in figure 1.5 after taking energy quantisation into account. Because of this quantisation, the molecules vibrate with an amplitude up to a cap marked by their energy level. By absorbing or emitting a photon, the molecule broadens or shortens its vibration.

$$= \left(n + 1 + \frac{1}{2} - n - \frac{1}{2}\right) hf = hf \tag{1.25}$$

This result is very important. This means that the energy transition from one level to the next is directly proportional to the vibrational frequency of the bond ν_b, which depends exclusively on the mass of the atoms and the strength of the bonds. Moreover, it is easy to show that the frequency of the applied radiation has to be exactly equal to that of the vibration:

$$\Delta E_{n\rightarrow n+1} = h\nu = hf \Rightarrow$$

$$\Rightarrow \nu = f \tag{1.26}$$

Then all the equations we developed in section 4. are still valid and we did not waste time with them.

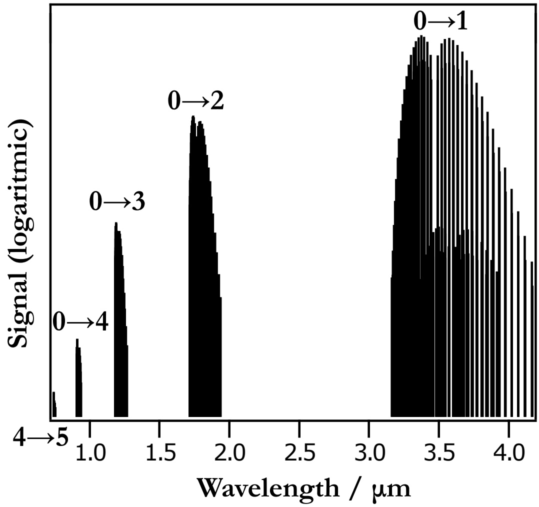


Figure 1.10: Fundamental absorption of hydrogen chloride and its first, second, third and fourth overtones. Note the perfect fulfilment of the relation of energies that we deduced in equation 1.27. Note also that the signal on the ordinate axis has a logarithmic scale and that the decrease in intensity at each overtone is very important. DOI source: 10.1088/0957-0233/14/9/318

We can also calculate the energy required for an overtone:

$$\Delta E_{n\to n+2} = \left((n+2)+\frac{1}{2}\right)hf - \left(n+\frac{1}{2}\right)hf =$$

$$= \left(n+2+\frac{1}{2}-n-\frac{1}{2}\right)hf = 2hf = 2\Delta E_{n\to n+1} \tag{1.27}$$

It is twice the energy required to go up from one level to the next.

Chapter 2

Instrumentation

In any type of spectroscopy, what is done is to see how much of the light incident on a sample at a given wavelength is absorbed by the sample. As this intensity can vary due to environmental variables such as the atmosphere present, the materials of the spectrometer windows, etc., it is compared with a reference, either with a double beam spectrometer that allows the spectrum of the sample and the reference to be measured at the same time or by taking a background, i.e., measuring first the spectrum of the reference and then that of the sample. In both cases, the spectrum of the reference (absorption of the environment) is subtracted from that of the sample (absorption of the environment + absorption of the sample), to obtain only the spectrum of the sample.

All types of spectroscopy always consist of three parts: a radiation source, a part to separate the wavelengths and the detector to measure the changes in the intensity of the radiation.

In the past, all infrared spectrometers were dispersive, i.e. the radiation, after passing through the sample, was separated into its different wavelengths or frequencies using a salt prism, a diffraction grating or both (figure 2.1), and the wavelength that reached the detector was selected with a slit. These devices were slow because to take a spectrum they had to sweep through all the required wavenumbers one by one. But nowadays everybody uses Fourier transform spectrometers, which obtain spectra with the help of an interferometer, because they are much faster (which allows measuring more spectra for averaging) and because, as they have fewer optical elements and no slits, the power of the radiation reaching the detector is higher and the signal-to-noise ratio (page 34) is much better.

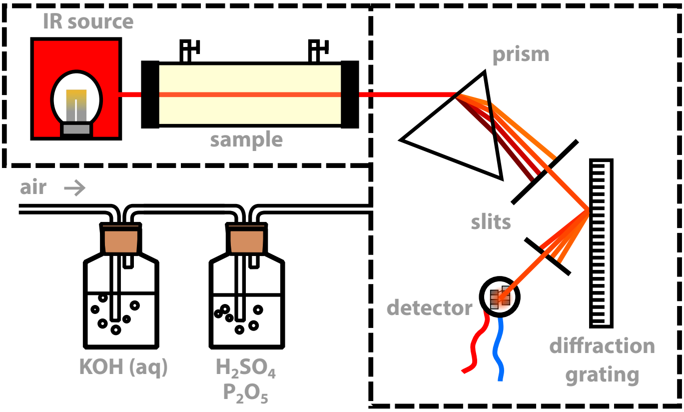


Figure 2.1: Diagram of the infrared dispersive spectrometer of Elmer Imes, a pioneer of infrared spectroscopy. By rotating the prism, he could control the wavelength of the radiation passing through the slit, which was then scattered by the diffraction grating.

§ 1. *Fourier transform spectrometers*

The Fourier transform is a mathematical operation that calculates the frequency with which the events of a periodic function are repeated. For example, if we have a cosine function that repeats every 2 seconds, by applying the Fourier transform we will obtain a function that will peak at 0.5 Hz. It is important to realise that by applying the Fourier transform we go from a function that depends on time to one that depends on frequency. If we have a more complex periodic function (figure 2.2), formed by the mixture of several periodic functions (for example, the sum of several cosine functions), by applying the Fourier transform we will obtain a function that will show a peak for each of the frequencies of the functions involved.

This simple mathematical operation can be applied to the study of infrared spectra. We apply a radiation to our sample which is a mixture of

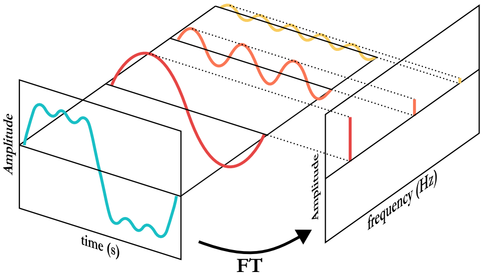


Figure 2.2: The Fourier transform in action. The function in time is composed of the sum of three cosines of different frequencies. By applying the Fourier transform we obtain a new function, whose variable is the frequency, composed of three peaks that appear at exactly the three different frequencies at which the three cosines oscillated.

waves of different frequencies of more or less the same amplitude. And after passing through the sample, some of these waves will be completely absorbed by the sample (the wave at a given frequency will disappear), others will be partially absorbed (the amplitude of these waves will be reduced) and others will pass through unaffected (and their amplitude will remain the same). Applying the Fourier transform to the wave that survives after passing through the sample, we obtain the same infrared spectrum that we would obtain with a dispersive spectrometer.

There is only one problem. Mid-infrared waves, for example, oscillate with a frequency between 12 and 120 THz, and there is no detector that even comes close to being able to respond to the variation in radiant power (twelve trillion times in one second) of such an extremely high frequency.

To solve the problem, we use a *Michelson-Morley interferometer*, which is an optical configuration that passes the radiation from the source through a beam splitter, which is a semi-transparent mirror that splits the light beam

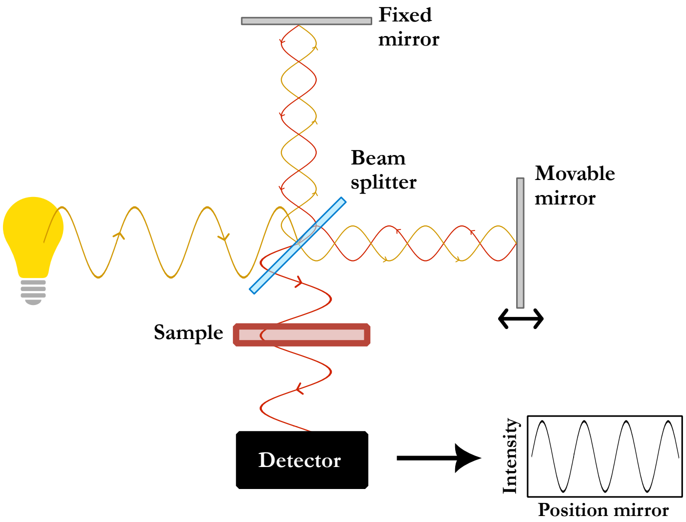


Figure 2.3: Diagram of a Michelson-Morley interferometer

into two beams of similar power (figure 2.3). Each of the beams created is then routed to two mirrors, one fixed and one movable, which return the light in the same direction in which it came, so that the beams are recombined again in the semi-transparent mirror and sent to the detector. If, because of the position of the movable mirror, the two rays arrive at the same time, they suffer a constructive interference, causing the detector to measure the maximum intensity. If the moving mirror is moved in such a way that its beam arrives exactly half a wavelength out of phase,[1] then, as the two waves combine, destructive interference occurs and no radiation will reach the detector. Therefore, as the mirror moves, radiation will appear and disappear in the detector periodically depending on its position and the wavelength of the radiation.

---

[1] In the infrared region this distance would be about 1 μm.

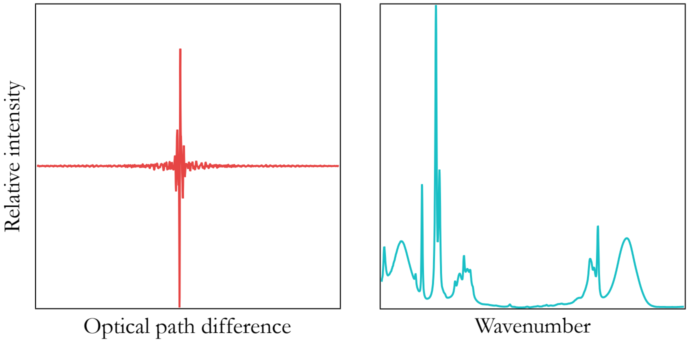


Figure 2.4: Interferogram and spectrum of ethanol obtained by performing the Fourier transform on it.

By passing the radiation through the interferometer we are effectively modulating its frequency, i.e. varying its value according to a signal, which in this case is the destructive constructive wave pattern. As the mirror moves at a constant speed v_m, every τ seconds it will go through a new cycle of the pattern:

$$v_m \tau = \frac{\lambda}{2} \tag{2.1}$$

Therefore, the frequency f of the signal in the detector will be:

$$f = \frac{1}{\tau} = \frac{2v_m}{\lambda} \tag{2.2}$$

We can exchange the wavelength for the frequency (equation 1.20, page 17) to see how the interferometer effectively, quickly and easily reduces the frequency of the radiation:

$$f = \frac{2v_m}{c}\nu \tag{2.3}$$

Thanks to the modulation of the interferometer, the frequency of the light is reduced by a factor equivalent to the speed of light. In other words, the frequency of the light arriving at the interferometer is of the order of 10^8 times smaller and by playing with the speed of the mirror it can be reduced even further, making it much easier to measure.

A Fourier transform spectrometer collects the signal at each mirror position.[2] This graph is known as an *interferogram*. By doing the Fourier transform of the interferogram we obtain the spectrum (figure 2.4).

In the next sections, we will look at the most common sources, beam splitters and detectors in today's infrared spectroscopy.

§ 2. *Sources*

Since bodies emit a large amount of infrared radiation when heated, materials that can be heated very much without being damaged are often used as sources of infrared radiation. The most common are:

- **Nerst glowers**: Ceramic rod made of rare earths oxides heated up to 2.200 K. They are used in the visible and near-infrared regions of the spectrum.

- **Tungsten halogen lamp**: Tungsten filament sealed into a envelope filled with a mixture of an inert gas and a small amount of iodine or bromine. This lamp is also used in the visible - near-infared regions (4.000 - 13.000 cm-1).

- **Globar**: Globar is an acronym of Glow bar. It is a silicon carbide rod heated up to 800 to 1,450 K that emits a strong radiation in the mid-infrared region of the spectrum.

---

[2] To know exactly where the mirror is at any given moment, modern spectrometers include a HeNe laser. Its wavelength, which is exactly 632.8 nm, creates a particular destructive constructive pattern that serves as a reference for the apparatus.

- **Mercury-vapor lamp**: Gas-discharge lamp that uses an electric arc through vaporized mercury. It is the most useful kind of lamp in the far-infrared region.

§ 3. *Detectors*

The simplest way to detect infrared radiation is to put your hand in its path. We will feel heat like when we are in front of a fire because the infrared radiation excites our molecules which start to vibrate and move and increase their temperature. This basic principle is behind many detectors. The most common ones are:

- **DLaTGS**: Acronym of deuterated Lanthanum α Alanine-doped Tri-Glycine Sulphate. It is a pyroelectric detector: it behaves as a capacitor whose efficiency depends on the temperature. Its spectral range depends on the material of its protective window:
  - KBr window: 12.000 – 250 cm^{-1}
  - CsI window: 12.000 – 130 cm^{-1}
  - Polyethylene window: 700 – 10 cm^{-1}
- **MCT**: Mercury cadmium telluride. This is a photoconductive detector: the incident light reduces the electrical resistance across two electrodes. It is 100 times more sensible than DLaTGS, but requires to be cooled constantly with liquid nitrogen. There are several types:
  - Narrow band: 12.000 – 850 cm^{-1}
  - Mid band: 12.000 – 600 cm^{-1}
  - Broad band: 12.000 – 420 cm^{-1}
- **InSb**: Indium antimonide is a crystalline compound that behaves as a semiconductor material, that is why it is used as a photoconductive detector. Requires liquid nitrogen cooling. Indium antimonide

detectors are sensitive to infrared wavelengths between 10.000 and 1.850 cm^{-1}.

- **InGaAs photodiode**: Similar to InSb, at room temperature its spectral range is between 12.800 – 5.800 cm^{-1}. Cooled it can reach a range of 12.800 – 4.000 cm^{-1}.

- **Silicon/Germanium bolometres**: This kind of detectors behave as thermistors, a device whose resistance is dependent on temperature. This detectors are used in far infrared spectroscopy thanks to their spectral range between 600 and 10 cm^{-1}. Their disadvantage is that they need to be cooled to 4 K with liquid helium, which is a very difficult substance to work with.

§ 4. *Beamsplitters*

To make a beamsplitter, a material is needed that does not absorb radiation in the region to be studied. A thin layer of metal is applied to this material, which will (partly) reflect the light, and also a protective layer is applied. As there is no material that is completely transparent in the entire infrared region, these are the most common materials and the regions in which it is possible to work with them:

- **CaF_2**: 15.000 – 1.200 cm^{-1}.

- **Quartz**: 25.000 – 9.000 cm^{-1}.

- **ZnSe**: 5.000 – 500 cm^{-1}.

- **Broadband KBr**: 10.000 – 400 cm^{-1}.

- **Standard KBr**: 7.500 – 370 cm^{-1}.

- **Silicon**: 400 – 60 cm^{-1}.

- **Mylar**: Mylar is the trade name for the polymer BoPET (biaxially-oriented polyethylene terephthalate). Its range depends on its thickness:
    - 25 μm: 120 – 30 cm^{-1}.
    - 50 μm: 55 – 10 cm^{-1}.

Beamsplitters made of KBr must be protected because it is a very hygroscopic material and quickly loses its optical properties in humid environments.

§ 5. *Parameters of a measurement*

Resolution

Spectral resolution is defined as the ability of the spectrometer to distinguish two wavenumbers that are very close to each other. In an FTIR spectrometer, the spectral resolution is determined by the distance travelled by the infrared radiation within the interferometer. The greater this distance, the greater the number of interference cycles that occur, which in turn allows better discrimination of nearby wavelengths. High resolutions (less than 2 cm^{-1}) are only used for gaseous samples, which are the only ones with such fine peaks.

Aperture

The spectrometers have a wheel with discs with holes in the centre of different apertures after the source. This is done to control the amount of radiation that is delivered to the interferometer. A wider aperture will allow more infrared radiation to be collected and increase the signal strength, but at the cost of losing resolution. The reason is that eventually the radiation is focused onto the detector and if the aperture is narrowed, more light travelling at an angle to the direction of the radiation is blocked. This angled light cannot be focused properly on the different parts of the spectrometer and generates distortions that reduce the resolution of the device. In most cases, it is recommended to use an intermediate numerical aperture, which provides an appropriate balance between signal strength and resolution. For example, a numerical aperture of 1.5 to 2 mm is considered a good choice for most applications. However, for samples that are highly absorbent or have a weak signal, a larger aperture can be used to increase the signal strength.

Scanner velocity and Gain setting

These two parameters control the time it takes to measure the interferogram and the sensitivity of the detector. The higher the sensitivity, the higher the signal, but also the noise. It is best to leave these parameters to be adjusted automatically if allowed by the software controlling the spectrometer.

§ *6. Signal-to-noise ratio*

One of the best ways to reduce the noise of a spectrum is to measure it many times and average the spectra obtained. However, it can be counter-intuitive because the noise does not reduce linearly with the number of measurements.

To demonstrate this, we can study the signal from an experiment (the absorbance of a spectrum at a particular wavenumber, for example) that we have obtained by averaging l measurements. We call these measurements S_1, S_2, S_3… up to the last one which will be S_l. The average of all the measured signals will be:

$$\bar{S} = \frac{\sum_{i=1}^{l} S_i}{l} \tag{2.4}$$

We can quantify the noise of the set of measurements through its dispersion around its mean, with the variance that is the average quadratic distance to the mean:

$$\sigma^2 = \frac{\sum_{i=1}^{l}(\bar{S} - S_i)^2}{l} \tag{2.5}$$

but the variance does not have the same units as the signal, so to define the noise N we use the standard deviation, which is the square root of the variance:

$$N = \sqrt{\sigma^2} = \sigma = \sqrt{\frac{\sum_{i=1}^{l}(\bar{S} - S_i)^2}{l}} \tag{2.6}$$

So we can compare the signal with its noise and define the signal-to-noise ratio, which tells us the quality of a measurement and is given by the quotient of the average and its standard deviation:

$$\frac{S}{N} = \frac{\bar{S}}{\sigma}$$

We repeat the experiment n times to see how the signal-to-noise ratio improves. The signal will now be

$$S = n\bar{S} \tag{2.7}$$

and, as when we repeat the experiment, we must add the variances, the noise will be:

$$N = \sqrt{n\sigma^2} = \sqrt{n}\sigma \tag{2.8}$$

so the total signal-to-noise ratio will be

$$\frac{S}{N} = \frac{n\bar{S}}{\sqrt{n}\sigma} = \sqrt{n}\frac{S}{\sigma} = \sqrt{n}\left(\frac{S}{N}\right)_i \tag{2.9}$$

That is, by repeating the experiments, the signal to noise ratio improves proportionally to the square root of the number of experiments done. In other words, to halve the noise of a measurement you have to make four measurements, to halve it again sixteen, to do it again 32 and so on.

Chapter 3

Techniques

In this chapter we discuss the different techniques for measuring infrared spectra. First we talk about the different ways to measure the interactions between radiation and matter. Then different ways to measure the spectra depending on the characteristics of the sample and the needs of the analysis.

§ *1. Absorbance, reflectance or transmittance*

When light hits a material, it can interact with it in four ways (figure 3.1). It can bounce off its surface, i.e., it is reflected. The amount of reflected light is not always the same and depends on the frequency of the light, its polarisation and the angle of incidence. If it manages to pass through the surface of the material, then it can either pass through it (be transmitted) or be partially or totally absorbed (opaque materials). In addition, if the material is not uniform and has particles of a certain size imbedded in it, then these particles will reflect light in multiple directions, scattering the light.

The scattered light, although very useful for other techniques, has to be avoided at all times when measuring an infrared spectrum as it distorts the measurements. To this end, if the sample is a liquid it must be filtered or if the sample is a KBr pellet (section 2., page 41) it must be ground as much as possible when preparing the pellet or increasing the pressure of the hydraulic press.

Reflected infrared radiation is complex and consists of two components: specular and diffuse. Diffuse light is light reflected from an irregular surface and can be used to measure the absorption spectrum of a sample (section 3., page 43). Specular radiation is used to measure the **reflectance** of smooth

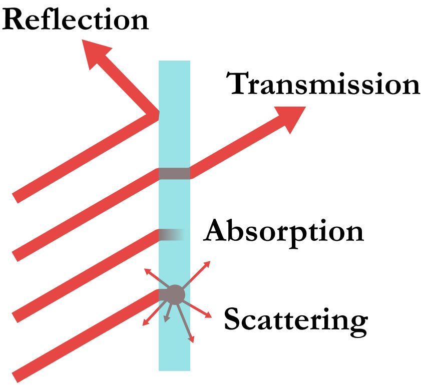


Figure 3.1: Types of light interaction with matter

surfaces,[1] which is the amount of radiation that is reflected by a surface. It is defined by the ratio of the applied intensity I_0 (measured by taking the background with a mirror) to that arriving at the detector I:

$$R = \frac{I}{I_0} \tag{3.1}$$

This quantity is usually expressed as a percentage. The measurement of reflectance has specific and limited applications, and usually, when one wants to know the physicochemical properties of a sample, one turns to measuring absorbance or transmittance spectra. When we do this, we must prepare the sample to reduce light reflection as much as possible and avoid distortions in the spectra.

Transmittance is the fraction of incident radiation that, at a specified

---

[1] Sometimes it is necessary to polish the surface in order to use specular radiation.

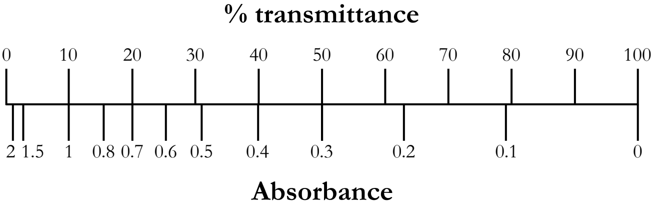


Figure 3.2: Scales showing the relationship between transmittance and absorbance

wavelength, passes through a sample. It is calculated with the formula:

$$T = \frac{I}{I_0} \tag{3.2}$$

where I_0 and I are the intensities applied to the sample and reaching the detector, respectively. To know the intensity applied to the sample, a background is usually taken.[2] T is usually expressed as a percentage multiplied by one hundred.

Once reflection and scattering are discarded, all radiation that is not transmitted by a sample is light that has been absorbed. So transmittance and **absorbance** are actually opposite concepts, as shown by the equation with which they are calculated:

$$A = \log_{10} \frac{I_0}{I} = -\log_{10} T \tag{3.3}$$

The reason why absorbance is used logarithmically is because, in this way, it depends linearly with sample concentration. This property of absorbing radiation of matter is known as the *Lambert-Beer law*. This law

---

[2] The background is usually taken without a sample, because even when cuvettes are used, due to the difficulties of working in the infrared, it is very difficult to prepare and place them in a reproducible way.

states that the absorbance of a sample is proportional to the concentration of the sample and the path length of light through the sample. Mathematically, the law is expressed as follows:

$$A = \varepsilon l c \tag{3.4}$$

where A is the absorbance of the sample, ε is the molar absorption coefficient (also called molar extinction coefficient), l is the path length of light through the sample (usually in centimetres) and c is the concentration of the sample. This law is in principle very useful for quantitative applications, but in the infrared, because the absorption bands are relatively narrow and the detectors are not very sensitive, concentration and absorbance rarely have a linear relationship and it is necessary to determine a calibration curve experimentally.

§ 2. *Transmission spectroscopy*

The transmission technique is the most common and consists of placing the sample directly in the path of infrared radiation.

If the sample is gaseous, a gas cell is usually used, which is a tube of a given length (as stated in the Lambert-Beer law, equation 3.3, the longer the length the higher the absorbance) with two windows transparent to infrared radiation at the ends (figure 3.3). The windows are usually made of halite (sodium chloride) glass, so great care must be taken when using them because they are fragile and degrade with moisture.

If the sample is a liquid, a dismountable liquid cell is usually used (figure 3.4). The windows are usually made of a material that is soluble in water, so they are almost always used to analyse organic liquids or samples dissolved in organic solvents. Because of the high absorption that these liquids usually have, the optical step (remember the Lambert-Beer law, equation 3.3) must be very short, usually less than one millimetre. To control the thickness of the sample, a Teflon spacer (in the figure 3.4 is lead) of known thickness is used between the two windows. Normally, for a sample, different

Figure 3.3: Gas cell. Gases are charged through one of the tubes on the side. The valves are closed when the partial pressure of the sample is adequate.

thicknesses must be tried until the most suitable one is found Care must be taken because, especially in the far infrared, light reflected from the inner faces of the windows can create constructive and destructive interference in the radiation waves and create distortions in the spectra. To solve this, it is necessary to increase the thickness of the cuvette or to use wedge-shaped cut windows.

If the sample is a solid, if it is a large, transparent body (such as a polymer) it can be placed directly in the path of the radiation avoiding as much as possible the reflection of light. Another option is to put it in suspension in a solvent. If the sample can be powdered, a potassium bromide (KBr) tablet can be prepared. Potassium bromide is an ideal material because it does not absorb infrared radiation and because it can be agglomerated under pres-

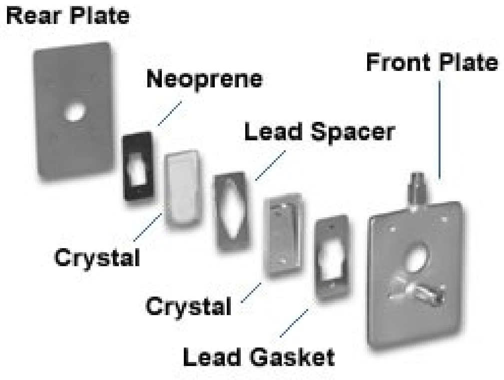


Figure 3.4: Components of a cell for liquids

sure. To prepare a KBr pellet, the following steps should be followed:

1. The appropriate amount of sample powder is weighed and mixed with KBr in a suitable ratio, usually in a ratio of 1:100 or 1:10, depending on the absorbance of the sample. It usually requires several attempts until the proper concentration is found.
2. The mixture is ground in an agate mortar to obtain a fine, homogeneous powder.
3. The KBr and sample mixture is placed in a hydraulic press and pressure is applied to form a pellet. The pellet must be transparent to the eye over its entire volume because otherwise it will scatter a lot of radiation. In addition, the pellet must also be thin enough to allow transmission of the infrared radiation through it, but not so thin that it will break during handling.
4. The pellet is placed in the holder and its spectrum is measured.

Care should be taken to carry out the whole process quickly to prevent the potassium bromide, which is hygroscopic, from absorbing too much moisture. If this occurs, the tablet will show small bands at the position of the water peaks (page 61).

§ 3. *Diffuse reflectance spectroscopy*

Diffuse reflectance spectroscopy is a technique that focuses infrared radiation onto the sample and collects the scattered radiation in all directions as it reflects off the sample. This is done with a very large concave mirror that collects the scattered radiation and focuses it onto the detector. This technique has the advantage that spectra can be measured with minimal sample preparation and is therefore commonly used in industrial processes. The sample can be a powder, a film or an irregular surface. Another advantage is that it allows measurement of infrared spectra of samples that are not transparent to infrared radiation.

On the disadvantages, the main one is that the diffuse radiation depends on the physical nature of the sample, with characteristics such as size and shape being very important. The variation of these parameters causes significant changes in the spectrum, thus contributing to a mixture of the physical and chemical information of the sample. Another disadvantage is that the intensity of the radiation scattered by the sample is usually very low and requires powerful light sources and multiple measurements.

The peaks obtained with this technique appear in the same positions as the absorption peaks of the sample, but their intensities change and generally the peaks that in the absorbance spectrum are of medium or small size have a higher intensity.

§ 4. *Attenuated total reflectance*

Attenuated total reflectance (ATR) is a method that allows the infrared absorption spectrum of a sample to be measured by simply placing it on

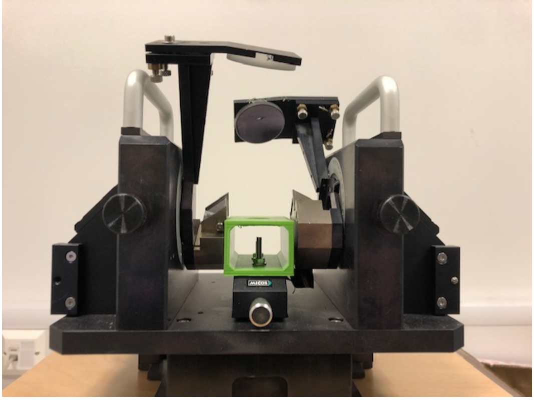


Figure 3.5: Photograph of a direct reflectance device. The angle of incidence can be controlled by the two mirrors at the top.

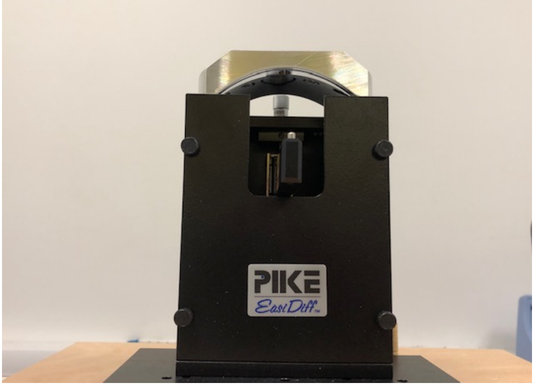


Figure 3.6: Photograph of a diffuse reflectance device. The upper dome is the one that collects the diffused radiation.

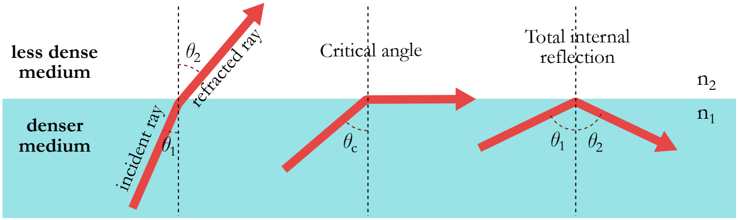


Figure 3.7: Light refraction depending on the angle. When the angle is sufficiently steep, internal reflection of the radiation occurs.

a crystal of a material with a high refractive index. This is much simpler than preparing KBr pellets or mounting a cell with liquids, which is why it is one of the techniques preferred by all spectroscopists. It is essential that the sample is in direct contact with the crystal and for example if its surface is irregular and is at a distance of a few micrometres from the crystal, its spectrum is no longer collected. Therefore, to measure the spectrum of solid samples it is necessary to press them against the crystal with the help of an anvil, which is usually included in the ATR accessories. This means that if the sample is fragile it will probably be irreparably damaged. This is the main drawback of this technique.

ATR spectroscopy is based on a property of electromagnetic radiation. When a light ray passes from a denser to a less dense medium, its angle will change (be refracted) at the junction separating the two media following *Snell's law* (figure 3.7):

$$n_1 \sin \theta_1 = n_2 \sin \theta_2 \tag{3.5}$$

where n_1 and n_2 are the refractive indices of the dense and less dense medium, respectively. $theta_1$ and $theta_2$ are the angles of incidence and refraction, respectively. Since the angle of refraction is always greater than the angle of incidence (the index of refraction depends on the density of the medium, so n_1 will always be greater than n_2), there is an angle of incidence at which the

Crystal	Colour	Spectral Range	n	pH	Hardness
Diamond	Transparent	50,000 – 50 cm^{-1}	2.4	1 – 14	9,000
ZnSe	Yellow	20,000 – 500 cm^{-1}	2.4	4 – 8	130
Ge	Silver-gray	5,000 – 550 cm^{-1}	4	1 – 14	780
Si	Silver-gray	400 - 60 cm^{-1}	3.4	1 – 12	965

Table 3.1: Properties of the most common ATR crystal materials

angle of refraction runs out of room to rotate further. This angle is called critical and it can be easily calculated because we know that θ_2 is 90°. Since the sine of 90°is 1, the *critical angle* is:

$$\sin\theta_1 = \frac{n_2}{n_1} \tag{3.6}$$

Therefore, whenever the light has an angle greater than the critical angle, it will be completely reflected on the inner surface of the dense material.

ATR instruments consist of a prism or a trapezoid of a high-refractive-index crystal transparent to infrared radiation. In addition, they are usually made of very hard materials to avoid damage during the measurement. The most common materials are listed in the table 4.. The radiation arrives on one side of the crystal at an angle of incidence that causes it to be internally reflected once or several times on its inner faces and then it is directed to the detector. Each time the beam undergoes internal reflection, an evanescent wave is created that passes through the interface and interacts with the sample. This evanescent wave is reflected by the sample and arrives at the detector, but on the way, part of the light has been absorbed by the sample and therefore, the light arriving at the detector contains the spectrum of the sample.

The penetration of the evanescent wave depends on the angle of incidence θ and the refractive indices of the crystal n_1 and the sample n_2. It can be calculated with the formula for the depth of penetration d that is defined as the distance required for the electric field amplitude to fall to e^{-1} of its

Figure 3.8: Diagram of an ATR crystal used to measure the IR spectrum of a mosquito.

value at the surface:

$$d = \frac{\lambda}{2\pi\sqrt{n_1^2 \sin^2\theta - n_2^2}} \tag{3.7}$$

λ is the wavelength of incident light in vacuum.

ATR spectra are similar, but not identical, to ordinary absorption spectra because the penetration is wavelength dependent.

To measure a sample, a measurement of air is usually used as a background. If the sample is liquid it is only necessary to make sure that the liquid covers the entire crystal and if it is solid it is necessary to make sure that the sample is in contact with the crystal surface by pressing with the anvil. If too much pressure is applied, the glass may fracture, so never exceed the factory-set contact pressure limit value. Always remove the pressure as soon as the measurement is finished. To clean the glass you can use a tissue soaked in water or ethanol (or first one and then the other to dry the glass).

CHAPTER 4

INFRARED SPECTRA

The infrared radiation region lies in the electromagnetic spectrum between visible radiation and microwave radiation. It is usually said to range from the end of the red colour around a wavelength of 750 nanometres (frequency 400 THz, 13,333 cm^{-1}), up to 1 millimetre (300 GHz, 10 cm^{-1}). This region is very wide and there are no detectors or emitters that cover it completely, so it is traditionally divided into three sub-regions: Near infrared, mid-infrared and far infrared.

§ *1. Near Infrared*

It comprises the radiation between the red edge of visible light and 4,000 cm^{-1}. In this region there are mainly peaks caused by overtones and combinations of fundamental vibrations. Overtones are when a molecule passes from its fundamental energy level to the second excited level (see section 8. in chapter 1 on page 22 and especially figure 1.10). And combinations are when two vibrations of a molecule share the energy of a photon (in other words, one photon excites two vibrational modes at the same time). As the vibrational energy levels are roughly spaced by the same energy, the frequencies of the overtones of a vibration are multiples of a fundamental frequency. For a molecule to rise to the second level is much less frequent than the fundamental vibration, which is why the absorption peaks are weaker. For a molecule to rise to a third level is even rarer, so these peaks are often indiscernible. While the overtones are very few and evenly spaced, even for a single molecule, a large number of combinations can be observed.

In organic molecules, most of the bonds between their atoms (C, N, O...) vibrate below 2000 $cm^{-1}$and their overtones are below the lower near-infrared limit. This means that most of the bands appearing in this region result from the overtones and combinations of the bonds of these atoms

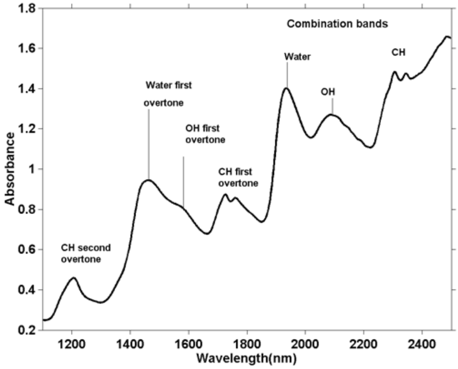


Figure 4.1: Spectrum of biscuit dough with the main areas of absorption identified. Source: A.M.C. Davies

with hydrogen.

There are two important advantages of near-infrared spectroscopy: the first is that it is the region whose spectra are taken the fastest, and the second is that it is the region in which it is easiest to work with reflectance spectroscopy (page 43), which does not require sample preparation, sample handling or sample destruction. This makes it a very popular technique in industry. As diffuse spectra are a mixture of the chemical and physical properties of the sample, creating a model requires many samples and hours of work.

§ 2. *Far Infrared*

It comprises the radiation between 400 $\mathrm{cm}^{-1}$and 10 cmcm^{-1}. In this region there are mainly peaks caused by the vibration of bonds involving atoms much heavier than carbon, so its main interest lies in the analysis of inorganic and organometallic species. Broad bands associated with collective vibrations (phonons) and rotational motions (librations) are also ob-

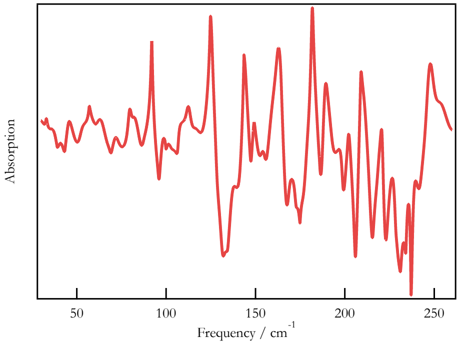


Figure 4.2: Far infrared spectrum of water vapour.

served in this region. As water vapour shows dozens of peaks in this region from its rotation (figure 4.2), any measurement requires completely dry atmospheres which makes it a very slow and cumbersome technique to use.

§ 3. *Mid Infrared*

The mid-infrared is by far the most widely used region for quantitative and qualitative applications. That is why, unless the other two regions are explicitly mentioned, when people talk about infrared spectra they refer only to this region. It extends between 4,000 and 400 cm^{-1}and most of the fundamental vibrations of the bonds in organic molecules occur in this range. The frequencies of these bonds can be calculated as indicated in section 8. of chapter 1, although it must be taken into account that the influence of neighbouring atoms to those of a bond can vary its frequency a little.

Roughly speaking, four different areas can be recognised in the mid-infrared spectrum depending on the frequency at which the stretching modes occur for each type of bond (figure 4.3).

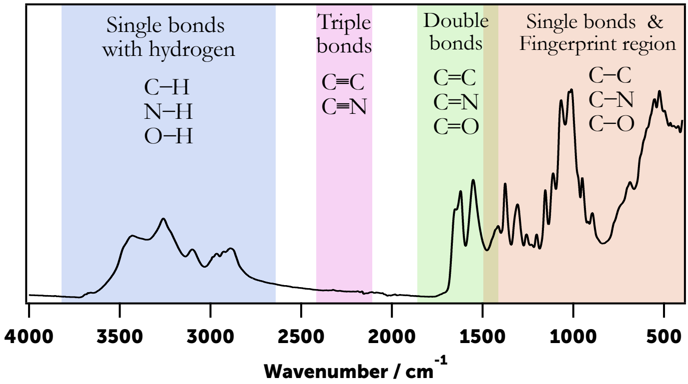


Figure 4.3: The four regions of the mid-infrared spectrum according to the position of the stretching vibrations of the most common organic bonds.

1. **Single bond region**: Single bonds between organic heavy atoms (C, N, O, S) have the lowest frequency and usually appear in the region between 1500 and 400 cm^{-1}. As their energies are very similar, there are many interactions between adjacent bonds and many combination peaks appear depending on the distribution of the bonds in the molecule. These combinations are so complex that interpreting the bands is extremely difficult. However, this makes the profile of these peaks characteristic for each molecule, so that with the help of a library of spectra it is possible to use this region for the unambiguous identification of pure compounds by comparison. This is why this region is also called the **fingerprint region**.

2. **Double bond region**: Between 1800 and 1450 cm^{-1}mainly double bonds between common organic atoms occur, because the force constant of these bonds is almost twice as high as that of single bonds (table 4., page 14).

3. **Triple bond region**: Similarly, it is between 2400 and 2200 centimetres where triple bonds appear. These bonds are rare, so peaks in this region are very revealing. They are also almost non-existent in biological samples, so compounds with the nitrile group ($-C\equiv N$) or the alkyl group ($-C\equiv C$) are often used to label biological samples.

4. **Bond with hydrogen region**: As we explained in the page 13 the great difference in mass between the hydrogen atom and the rest of the organic atoms (C, N, O, S) makes the stretching vibration frequency much higher than that of the simple bonds between these heavier organic atoms. This is why this region is between the higher frequency limit of the mid-infrared and 2600 cm^{-1}.

CHAPTER 5

INTERPRETATION OF INFRARED SPECTRA

Although (mid) infrared spectroscopy is not usually used exclusively to identify pure organic compounds, the peaks associated with each organic functional group are so characteristic that they can be of great help in understanding the composition of the molecule. Here are the tricks that every spectroscopist should know to interpret a spectrum with the naked eye.

To assign each peak, it is necessary to consult an infrared tables book or to calculate the vibrations of the molecule with a programme such as Gaussian. The most important thing is to differentiate between the intensity of the peaks (weak, medium, intense) and their width (narrow or wide peaks) and to know the most characteristic frequencies of the most common bonds.

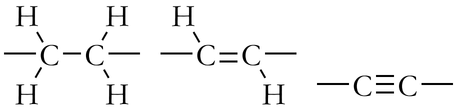
§ *1. Peak intensity*

In the 6. section of the first chapter (page 18) we saw that the higher the dipole moment of a bond, the more it absorbed infrared radiation. Translated to an infrared spectrum, this means that the intensity of the peaks depends on the dipole moment and the more intense the dipole of the associated bond, the more radiation it will absorb, and the more intense the absorption band will be. Highly polar bonds such as the carbonyl bond (C=O , page 63) produce intense bands. Low-polar bonds (formed by atoms of similar electronegativity, such as -C-N-, page 66) produce bands of medium intensity. Bonds formed by two equal atoms, if they are part of an asymmetric molecule, such as, for example, the alkyl bond of $HC{\equiv}C{-}CH_3$, the asymmetry will make one side slightly richer in electrons and generate a small dipole that produces a weak peak. If this kind of bond forms part of a symmetric molecule, as for example in the alkyl bond of $H_3C{-}C{\equiv}C{-}CH_3$, there will be no dipole and therefore no peak.

§ 2. *Peak width*

The width of the peaks depends on the freedom of the molecules to vibrate. The infrared spectra of gas-phase molecules are composed of very thin peaks because the space between molecules is very large and, except when they collide, they can vibrate without being influenced by their neighbours. This causes them all to vibrate at the same frequency and, therefore, the peak is very thin. However, in solid or liquid samples, molecules are surrounded by their neighbours and are continuously interacting with them, which causes their vibration to be altered depending on the space available to them at any given moment. Therefore, instead of vibrating at a single frequency, they are distributed over a range of frequencies (which is why the peaks are usually Gaussian bell-shaped) and why the peaks are wider. In addition, the vibrations will be influenced by the intermolecular forces or weak bonds they form with the surrounding molecules. The strongest intermolecular force is the hydrogen bond that forms between atoms that have a hydrogen atom bonded to an electronegative atom such as O or N. This is why the stretching bands of alcohols, for example, are so wide. The vibration of a carboxylic group (C=O, very polar) in an organic molecule (usually not very polar) has hardly any chance to undergo intermolecular forces and therefore usually produces very thin peaks.

§ 3. *Alkanes, alkenes and alkynes*



Hydrocarbons are compounds made only of carbons and hydrogens. Hydrocarbons that do not form cycles are called aliphatic hydrocarbons. Hydrocarbons are characterised by a very intense double or triple peak associated with C–H stretching just below 3,000 cm^{-1}. It is very important to draw an imaginary line at 3,000 cm^{-1}, because if there is a narrow peak above that frequency it means that there is a double bond (alkene group: 3,080 cm^{-1}stretch =C–H) or triple bond (alkyne group: 3,300 cm^{-1} stretch ≡C–H). These peaks are weaker than those of the single bonds and the peak of the double bond may be hidden at the base of the intense peak of a single bond. Alkenes and alkynes also show a weak and very narrow peak corresponding to the stretching –C=C– or –C≡C– around 1,600 and 2,100 cm^{-1}, respectively.

If you see a peak at 2,100 cm^{-1}, even if it is weak, you should not ignore it. It is probably the only small peak that is not ignored for its size because it is your best evidence that an alkyne group is present.

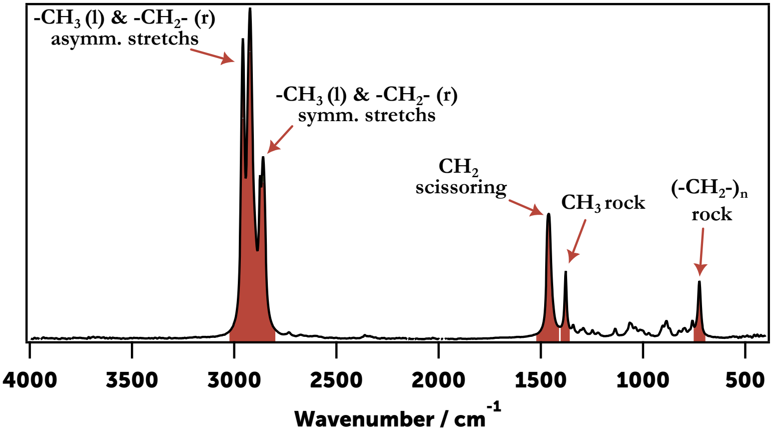


Figure 5.1: Alkanes: Infrared spectrum of hexane.

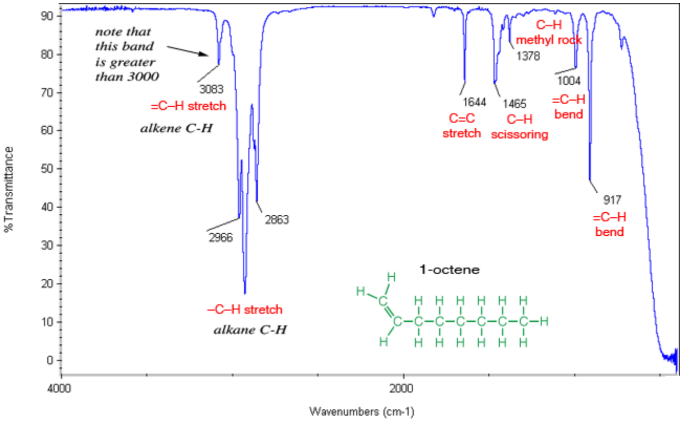


Figure 5.2: Alkenes: Infrared spectrum of 1-octene.

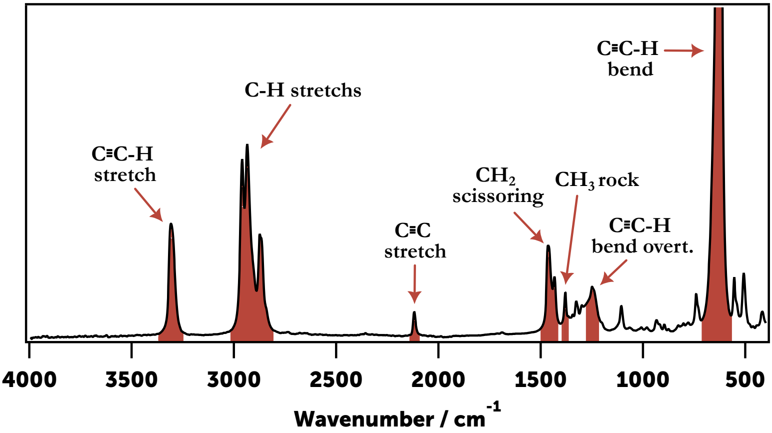
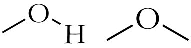


Figure 5.3: Alkynes: Infrared spectrum of 1-hexyne.

§ 4. *Alcohols and ethers*



The presence of alcohols is easy to identify by the exaggerated broadening of the –O–H stretching band located between 3,000 and 3,700 cm^{-1} due to the hydrogen bonds that their hydrogens can form. They also show the C–O stretches which usually appear as an intense doublet between 1,260 and 970 cm^{-1}.

Ethers that are organic compounds that are commonly used as solvents. They contain an oxygen atom bonded to two alkyl groups. They show the peak of the C–O stretches but do not have the intense band of the –O–H stretching.

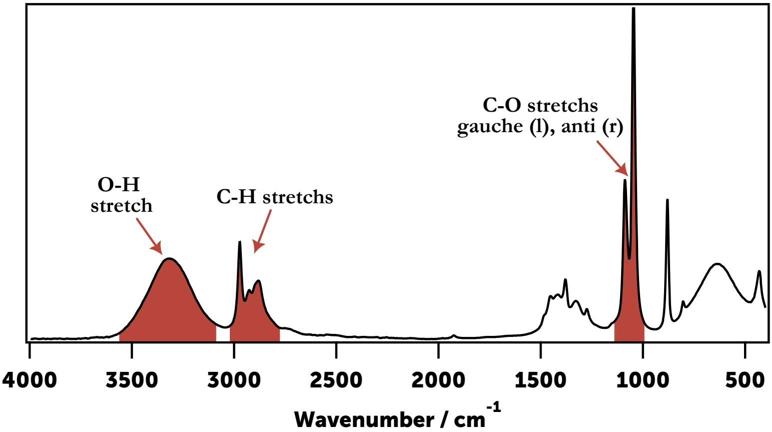


Figure 5.4: Alcohols: Infrared spectrum of ethanol.

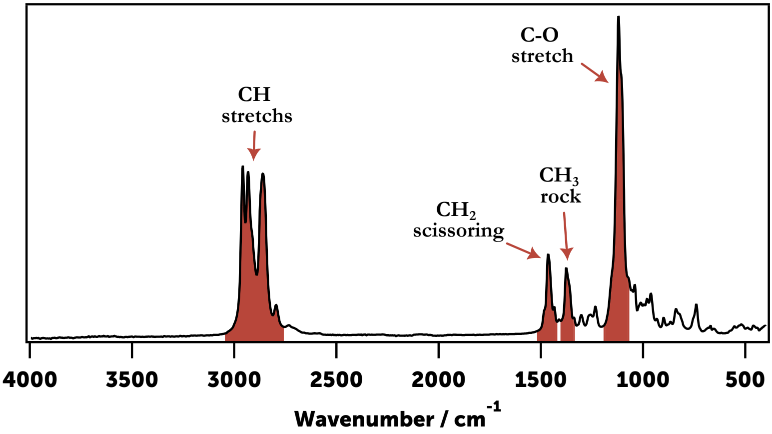
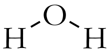


Figure 5.5: Ethers: Infrared spectrum of dibutyl ether

§ 5. *Water*



Water molecules are so polar that the three bands they have are so intense that if a sample contains water their peaks are completely hidden by it. The three peaks are the intense broad peak of the –O–H stretch, a broad combinatorial peak at 2,050 cm^{-1}, a thin peak at 1,650 cm^{-1} due to the bending mode and a band growing towards the low frequency positions that is due to the impeded rotation of the water molecules (libration).

The explanation of the infrared bands of liquid water in the near- (figure 5.7) and mid-infrared (figure 5.6) is:

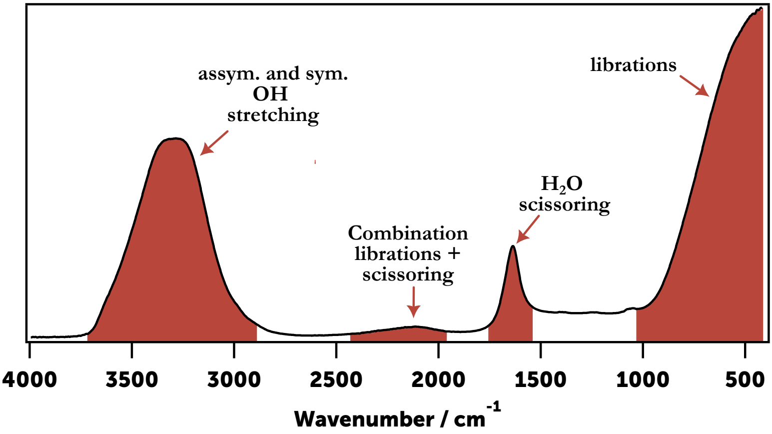


Figure 5.6: Mid-infrared spectrum of liquid water

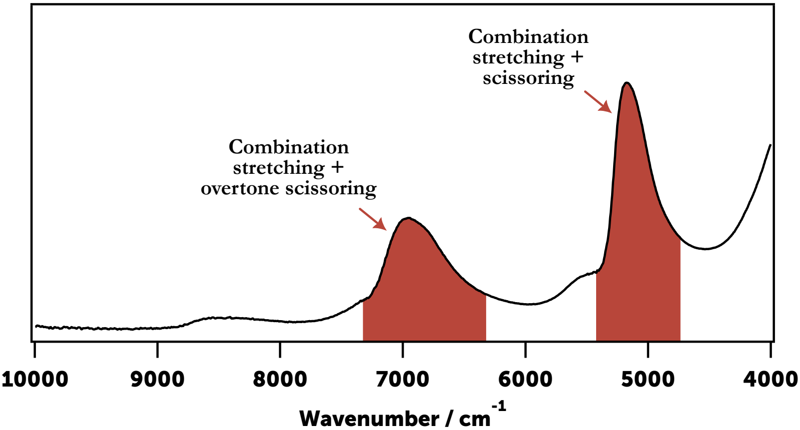
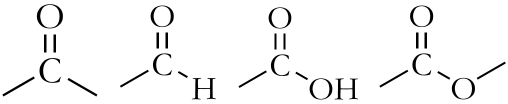


Figure 5.7: Near-infrared spectrum of liquid water. Take into account that the intensity of this spectrum is five times weaker than the mid-infrared spectrum of liquid water.

§ 6. *Ketones, aldehydes, carboxylic acids, and esters*



Compounds with the carbonyl bond C=O are also easy to identify because they are the only ones that show a very intense narrow peak in the region between 1,840 – 1,650 cm^{-1}. To distinguish between ketones, aldehydes and carboxylic acids one looks at the high-frequency part. If an extra peak of intermediate intensity appears in the C–H stretching region around 2,720 cm^{-1}, it is the special C-H bond of an aldehyde. And if there is a band like the alcohols but much wider between 2,800 and 3,500 centimetres, it is a carboxylic acid. An ester shows the peak of the carbonyl bond and the relatively intense C–O–C stretching band or bands that ethers show in the region between 1,260 and 970 cm^{-1}.

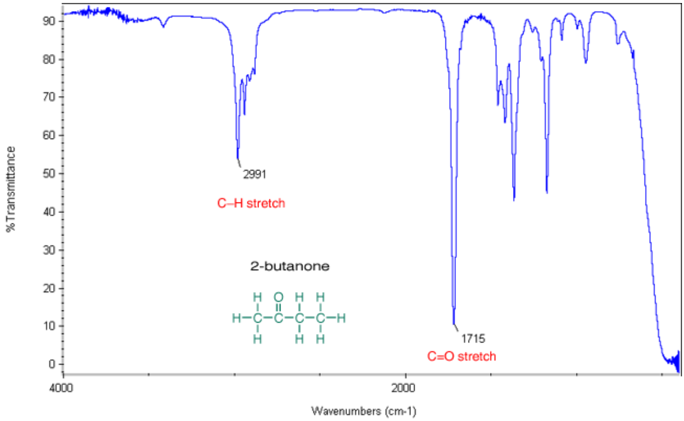


Figure 5.8: Ketones: Infrared spectrum of 2-butanone

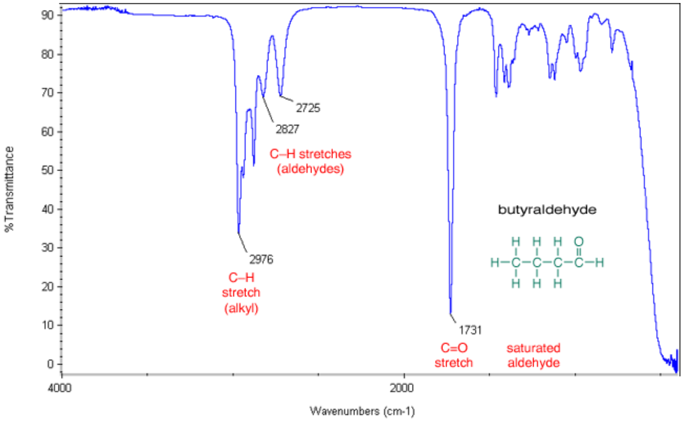


Figure 5.9: Aldehydes: Infrared spectrum of butanal

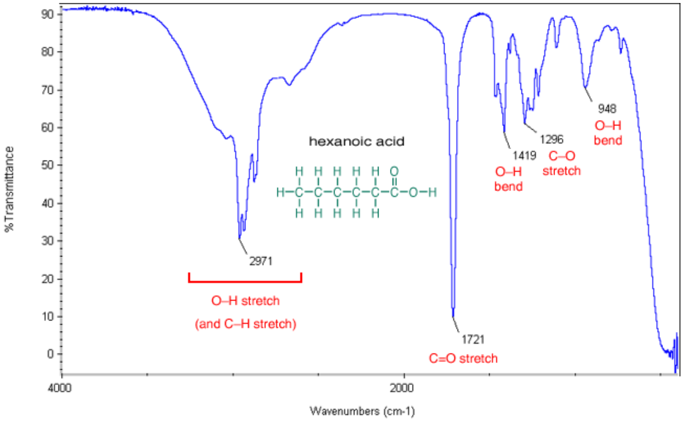


Figure 5.10: Carboxylic acids: Infrared spectrum of hexanoic acid

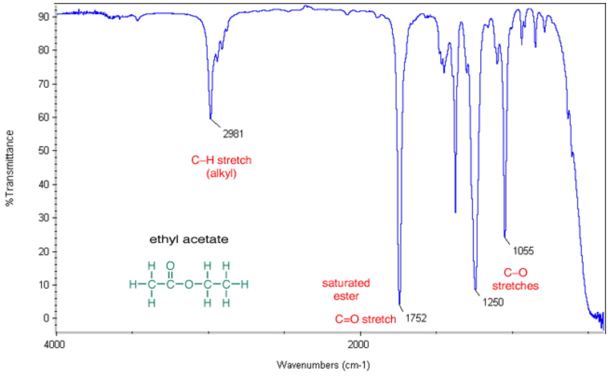
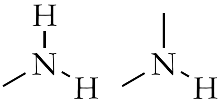


Figure 5.11: Esters: Infrared spectrum of ethyl acetate

§ 7. *Primary and secondary amines*



The primary amines show their -N–H stretching band in the same region as the alcohols, but their band is sharper and as there are two hydrogens, it is double. In addition, they show a very intense and narrow peak of the H–N–H bond bending mode in the region between 1,650–1,580 cm^{-1}. It is right next to where the also very intense and narrow peak of the carbonyl bond appears so here we draw another boundary at 1,650 cm^{-1}. If the peak is above the boundary it is a carbonyl bond, if it is below it is a primary amine. Secondary amines show their -N–H stretching band in the same region as primary amines but, as they only have one hydrogen, they show a single band.[1]

---

[1] One way to remember this is that a primary amine band looks like a molar and a secondary

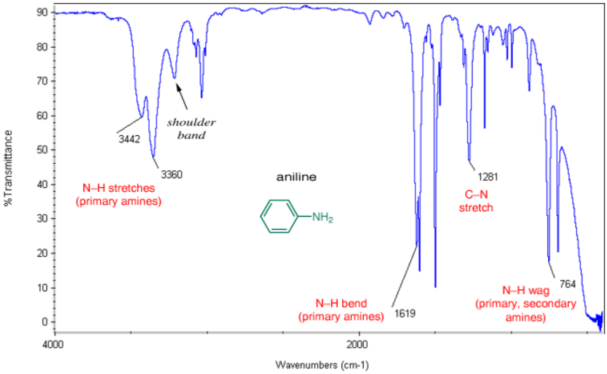


Figure 5.12: Primary amines: Infrared spectrum of aniline

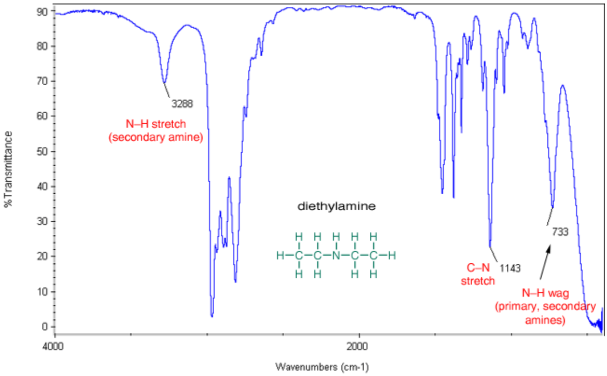
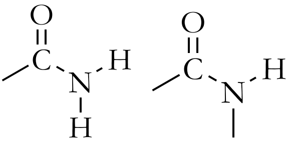


Figure 5.13: Secondary amines: Infrared spectrum of diethylamine

---

amine band looks like a canine.

§ 8. *Primary and secondary amides*



Amides show the N-H stretching band and the fine and intense peak of the carboxylic bond stretching. If the amide is primary, this peak is accompanied on its lower frequency side by the H–N–H bond bending peak.

Secondary amides are the main components of proteins, which we will study in more detail in the "protein" section of the next chapter.

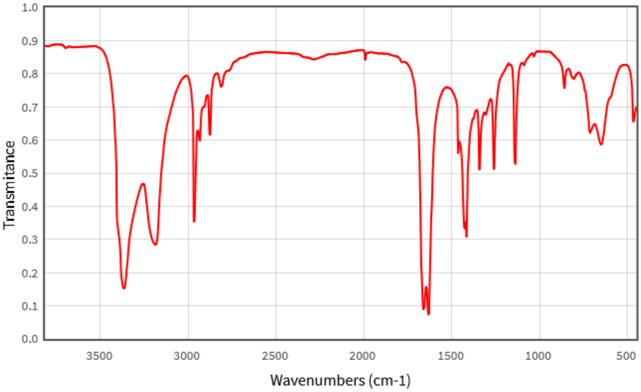


Figure 5.14: Primary amides: Infrared spectrum of butylamide

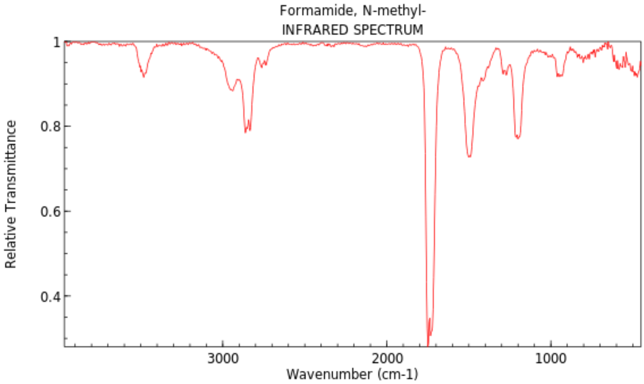


Figure 5.15: Secondary amides: Infrared spectrum of N-methyl formamide

CHAPTER 6

INFRARED SPECTRA OF INTEREST TO ENTOMOLOGISTS

In this chapter we can use what we have learned so far to better understand the most common spectra that an entomologist may face.

§ *1. Proteins*

Proteins are large molecules that are made of amino acids. Amino acids are organic molecules composed of an amino group (–NH_2), a carboxyl group (-COOH) and a variable side chain (also called an R group), which is specific to each amino acid. There are 20 common amino acids that are used to build proteins in living things. Amino acids are linked by a peptide bond that is formed through a condensation reaction in which the carboxyl group of one amino acid joins with the amino group of the next amino acid to form an amide. This is why amide peaks (section 8. of the last chapter, page 68) dominate the infrared spectrum of proteins (figure 6.1). They are so important that the peaks are given specific names: Amide A, which is the name given to the -N–H stretching band; Amide I, which is the carboxylic group stretching band; Amide II, which is the asymmetric bending band of the primary amine; and Amide III, which is the symmetric bending band (figure 6.2).

The peptide bond is rigid and flat due to the resonance structure that forms, so the conformation of the proteins greatly alters the appearance of the amide bands. In a pure protein sample, large differences between the wavenumber of amide band I and amide band II suggests a helix conformation. The opposite would be a beta-sheet conformation. When the protein is dominated by a random coil conformation its peaks are usually broad (figure 6.3)

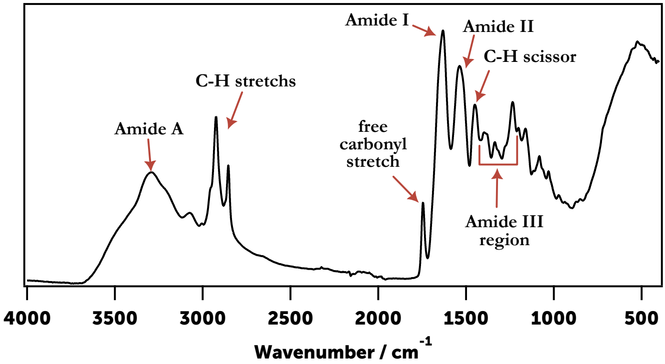


Figure 6.1: Spectrum of a protein (collagen) with the bands of the amides shown: Amide A, Amide I (1650 cm^{-1}), Amide II (1550 cm^{-1}), and Amide III (1400 – 1200 cm^{-1})

Other significant bands in the spectrum of a protein are the band of the -O–H stretching of the alcohols on which the amide A band grows, the band of the carbonyl groups that do not belong to the peptide bond (1745 cm^{-1}) or the band of the C-H scissoring that appears between the amide II and amide III bands (1455 cm^{-1}).

§ 2. *Waxes and fatty acids*

The waxes and fatty acids that usually coat insect cuticles are very simple from the point of view of infrared spectra as they are made of long aliphatic chains and carboxylic acid (the fatty acids) or ester (the waxes). Therefore, they always have three intense peaks which are the two C–H stretching peaks just below 3000 cm^{-1}(if there are peaks above this boundary it means that the wax or fatty acids have unsaturations (double bonds)) and the peak of the carbonyl bond C=O around 1750–1735 cm^{-1}. Other common peaks

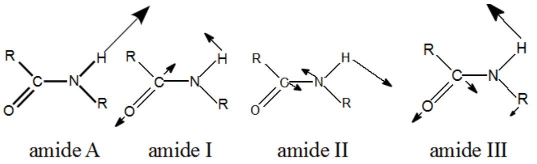


Figure 6.2: Vibration mode behind each band of the characteristic protein spectrum.

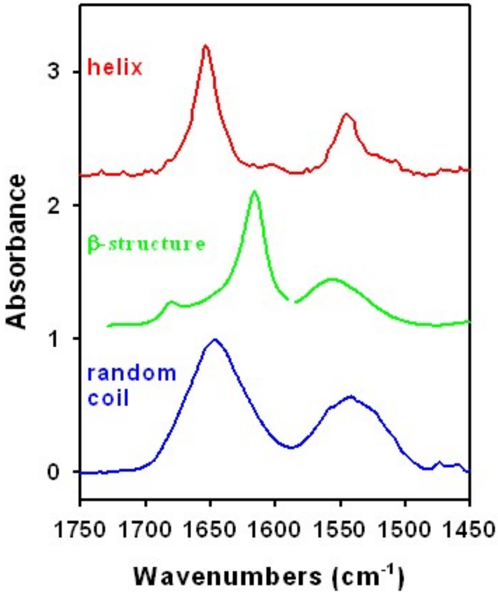


Figure 6.3: Change in amide bands I and II depending on the conformation of the protein.

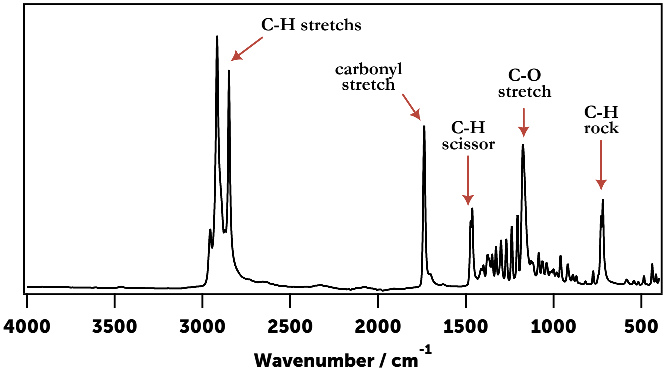


Figure 6.4: IR spectrum of a wax (arachidyl dodecanoate)

are the C–O stretches which appear as two bands in the 1330–1050 cm^{-1} region: a more intense, higher wavenumber, band corresponding to asymmetric stretching and a lower wavenumber, less intense band corresponding to symmetric stretching. Sometimes, as in figure 6.4, this band could be pretty weak.

§ 3. *Chitin*

Chitin is a naturally occurring polysaccharide that forms the exoskeleton of many insects. It is a homopolysaccharide composed of N-acetylglucosamine units linked by beta-1,4-glycosidic linkages. It is very similar in structure to cellulose, which has the same type of glycosidic linkage, except that the alcohol at carbon 2 is replaced by an acetyl amine. This chemical structure gives it unique properties, such as its resistance to degradation by digestive enzymes, the toughness and rigidity it gives to arthropod exoskeletons and mollusc shells, and its ability to form biodegradable films.

Chitin, like cellulose, is very rich in -C–O bonds (from the alcohols and

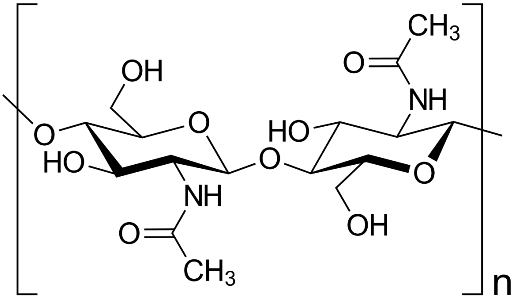


Figure 6.5: Structure of chitin.

the hemiacetal group of its monomer), which is why the C-O stretching region is so intense and rich. In addition, the vast majority of the carbons are in the form of cycles, so the C–H stretching band is quite weak. The carbonyl group of the acetyl appears at approximately 1670 cm^{-1}.

§ *4.* ***Water vapour and carbon dioxide***

Water vapour and carbon dioxide are the two atmospheric constituents that absorb infrared radiation (see section 6. from chapter 1) and are the main intruders of an infrared spectrum. As in a laboratory environment their concentration changes over time due to breathing of operators or ventilation with air of different characteristics, after taking a background as time passes, their peaks usually appear and start to become more intense, either positive (if their concentration since taking the background increases) or negative (if it decreases).

The spectrum of water vapour has three bands: 4000 – 3400 cm^{-1}, which is the O–H stretching; 2000 - 1300 cm^{-1} which is the scissors bending mode of the water molecule and below 700 cm^{-1} is the beginning of the rotational part of its spectrum.

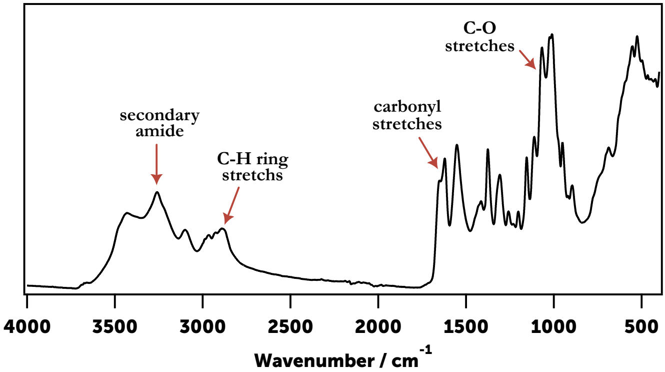


Figure 6.6: IR spectrum of chitin

The spectrum of carbon dioxide also has three bands in the mid-IR region: 2420–2250 cm^{-1} asymmetric stretch, 770 – 500 cm^{-1} scissors bend mode, and 3800–3550 cm^{-1}, that is a combination band of the asymmetric stretch and the symmetric stretch, that happens with a frequency of 1388 cm^{-1}, but it doesn't appear in the spectrum because this mode doesn't change the dipolar moment of the molecule (page 19)

Their appearance in the form of two bands filled with very narrow peaks is due to the fact that as they are gases, it is possible to see in detail the peaks associated with molecular rotations, which require less energy, and at the position of one band we can see several transitions between different rotational energy levels. These peaks may not be well defined if the resolution is low.

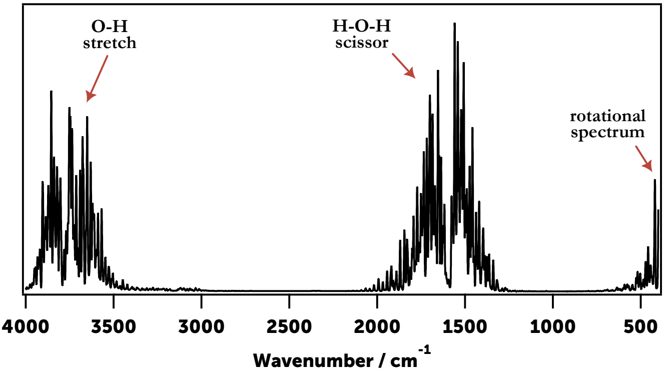


Figure 6.7: Infrared spectrum of water vapour

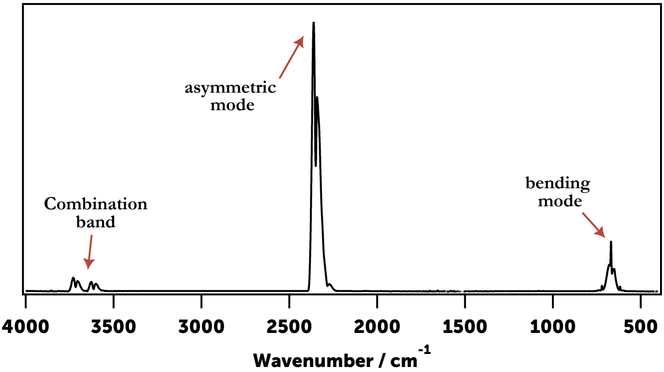


Figure 6.8: Infrared spectrum of carbon dioxide