CHAPTER 29: INTRODUCTION TO CHROMATOGRAPHY AND SPECTROSCOPY

Organic and Biochemistry Supplement to Enhanced Introductory College Chemistry

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Chapter 29 Contents

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College Chemistry (https://ecampusontario.pressbooks.pub/orgbiochemsupplement/) to access the complete book, interactive activities and ancillary resources.

In this chapter, you will learn about:

- The process of chromatography
- Various chromatography methods and technologies
- The process of spectroscopy
- Various spectroscopy methods and technologies
- How to identify compounds based on their IR, MS, NMR and UV-Vis spectra

To better support your learning, you should be familiar with the following concepts before starting this chapter:

- Knowledge of functional groups and their physical properties (Chapter 19: Organic Chemistry)
- Naming and structures of simple organic molecules

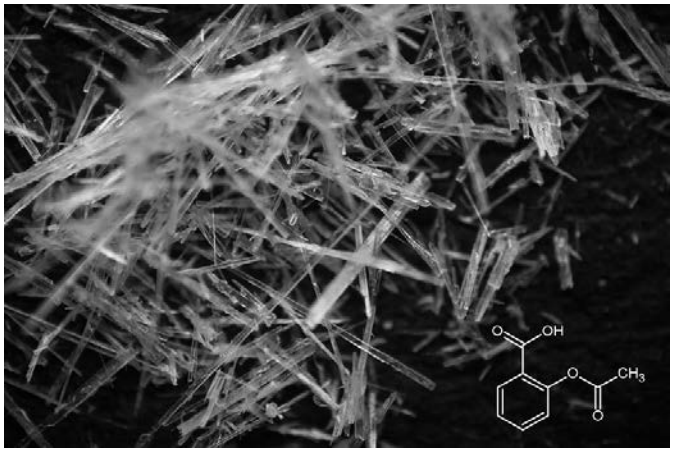

Figure 29.0a. Acetylsalicylic acid crystals and their chemical structure. (Credit: Image by Steve Mike Neef, CC BY-SA 3.0)

How do scientists know the structure of a compound like that of acetylsalicyclic acid in Figure 29a? How do scientists know what elements are in that compound? How do scientists separate one compound from a mixture?

Watch Craze Sports Supplement Reportedly Contains Meth-Like Compound on YouTube (2 min) (https://youtu.be/0FCCszlsMWs)

In the video, scientists identified a potentially harmful compound in a sports supplement. They used tools and processes to detect components of a mixture, separate the mixture and identify elements and structures of compounds.

These tools are broadly classified as chromatography methods and spectroscopy methods.

Chromatography is a method by which a mixture is separated by distributing its components between two phases. The stationary phase remains fixed in place while the mobile phase carries the components of the mixture through the medium being used. Because of the differences in factors such as the solubility of certain components in the mobile phase and the strength of their affinities for the stationary phase, some components will move faster than others, thus facilitating the separation of the components within that mixture.

Spectroscopy is used to determine the structure of a compound. Electromagnetic radiation of a specified range of wavelengths is allowed to pass through a sample containing a compound of interest. The sample molecules absorb energy from some of the wavelengths, and as a result jump from a low energy 'ground state' to some higher energy 'excited state'. Other wavelengths are not absorbed by the sample molecule, so they pass on through. A detector on the other side of the sample records which wavelengths were absorbed, and to what extent they were absorbed.

In this chapter, a basic introduction to several chromatography and spectroscopy techniques will be explored.

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29.1 CHROMATOGRAPHY BASICS

Learning Objectives

By the end of this section, you will be able to:

- Identify the purpose of chromatography.
- Describe the function of the stationary and mobile phases.

A useful set of techniques for separating mixtures is called **chromatography**. There are many different chromatography methods. Each has its own purposes and limitations.

All forms of chromatography work on the same general principle. There is always a stationary phase which does not move and a mobile phase which does. The various components in the mixture being chromatographed separate from each other because they are more strongly held by one phase or the other. Those which have the greatest affinity for the mobile phase move along the fastest.

As the mobile phase moves along the stationary phase, the sample partially dissolves and travels along the stationary phase. Different substances will travel different distances along the stationary phase. The distance that a substance will travel depends on how strongly it adheres to the stationary phase (a process called adsorption) versus how much time it spends dissolved in the mobile phase. The more a substance adsorbs, the less it dissolves and the less it moves along the plate. The less a substance adsorbs, the more it dissolves and the farther it travels. The process is continued until a good separation is created. In this manner, a mixture of substances may be separated.

The stationary phase acts as a constraint on many of the components in a mixture, slowing them down to move slower than the mobile phase. The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases. Because of the differences in factors such as the solubility of certain components in the mobile phase and the strength of their affinities for the stationary phase, some components will move faster than others, thus facilitating the separation of the components within that mixture.

Perhaps the simplest of these techniques to describe is paper chromatography (Figure 29.1a.), which is described in the next section.

Figure 29.1a. Picture of paper chromatography of various marker ink. (Credit: Photo by Amitchell125, CC BY-SA 4.0)

The development of chromatography is one of the major revolutions in technique in the history of chemistry, comparable to that which followed the development of an accurate balance. Separations which were previously considered impossible are now easily achieved, sometimes with quite simple apparatus. This technique is particular essential to the science of biochemistry, in which complex mixtures are almost always encountered. In the field of environmental chemistry, chromatography has helped us separate and detect very low concentrations of contaminants like DDT or PCB (polychlorinated biphenyls). The major drawback to chromatography is that it does not lend itself to large-scale operation. As a result, it remains largely a laboratory, rather than an industrial, technique for separating mixtures.

Watch Synthesis and Column Chromatography: Crash Course Organic Chemistry #25 on YouTube (12 mins) (https://youtu.be/IGC5J_7gkKg) *Chromatography starts about 2 min mark

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29.2 THIN LAYER (TLC) AND PAPER CHROMATOGRAPHY (PC)

Learning Objectives

By the end of this section, you will be able to:

- Describe the purpose and procedure of paper chromatography.
- Calculate the R_f value.
- Describe the purpose and procedure of thin layer chromatography.

Paper Chromatography (PC)

In paper chromatography, the stationary phase is a very uniform absorbent paper. The mobile phase is a suitable liquid solvent (typically, water) or mixture of solvents. Here's a quick paper chromatography experiment you can try at home using water soluble markers (or food colouring), paper towel (or coffee filter) and water.

Watch Marker Pen Chromatography – FLEET Centre Home Science on YouTube (2 mins) (https://youtu.be/u_9nICZlVmM)

Setting up a Paper Chromatography Experiment

Suppose you have three blue pens, and you want to find out which one was used to write a message. Samples of each ink are spotted on to a pencil line drawn on a sheet of chromatography paper. Some of the ink from the message is dissolved in the minimum possible amount of a suitable solvent, and that is also spotted onto the same line. In Figure 29.2a., the pens are labeled 1, 2 and 3, and the message ink as M.

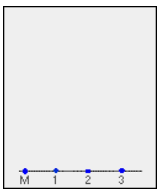


Figure 29.2a. Setup of paper chromatography (credit: *Chromatography* , CC BY-NC 4.0).

The paper is suspended in a container with a shallow layer of a suitable solvent or mixture of solvents in it. It is important that the solvent level is below the line with the spots on it. The Figure 29.2b. doesn't show details of how the paper is suspended because there are multiple ways of doing it.

The reason for covering the container is to make sure that the atmosphere in the beaker is saturated with solvent vapour. Saturating the atmosphere in the beaker with vapour stops the solvent from evaporating as it rises up the paper.

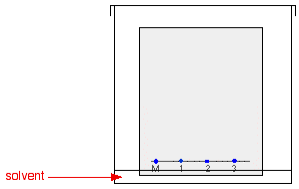


Figure 29.2b. Placement of solvent in paper chromatography (credit: *Chromatography* , CC BY-NC 4.0).

As the solvent slowly travels up the paper, the different components of the ink mixtures travel at different rates and the mixtures are separated into different coloured spots. Figure 29.2c. shows what the plate might look like after the solvent has moved almost to the top.

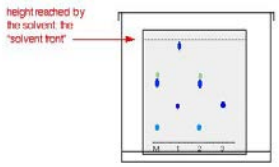


Figure 29.2c. Paper chromatography result after solvent (mobile phase) moves (credit: *Chromatography* , CC BY-NC 4.0).

It is fairly easy to see from the final result that the pen that wrote the message contained the same dyes as pen

2. You can also see that pen 1 contains a mixture of two different blue dyes – one of which might be the same as the single dye in pen 3.

R_f values

Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the base line. The distance travelled relative to the solvent is a constant for a particular compound as long as you keep everything else constant – the type of paper and the exact composition of the solvent, for example.

The distance travelled relative to the solvent is called the R_f value. For each compound it can be worked out using the formula:

$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

(credit: *Chromatography* , CC BY-NC 4.0).

Example 29.2a

Calculate the R_f value for a paper chromatography result if one component of a mixture travelled 9.6 cm from the base line while the solvent had travelled 12.0 cm.

Solution

The R_f value for that component is:

$$R_f = \frac{9.6}{12.0} = 0.80$$

(credit: *Chromatography* , CC BY-NC 4.0).

In the example we looked at with the various pens, it wasn't necessary to measure R_f values because you are making a direct comparison just by looking at the result.

You are making the assumption that if you have two spots in the final result which are the same colour and have travelled the same distance up the paper, they are most likely the same compound. It isn't necessarily true of course – you could have two similarly coloured compounds with very similar R_f values.

In some cases, it is possible to see the results of paper chromatography because the spots are coloured. Other

times, the spots may not be visible. However, it may be possible to make the spots visible by reacting them with something which produces a coloured product. In Figure 29.2d., a paper chromatography experiment is run with different amino acids. After the experiment is run, the paper is sprayed with a solution of ninhydrin. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple.

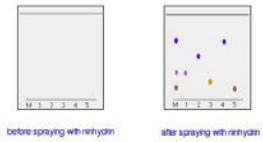


Figure 29.2d. The left-hand diagram shows the paper after the solvent front has almost reached the top. The spots are still invisible. The second diagram shows what it might look like after spraying with ninhydrin (credit: *Chromatography* , CC BY-NC 4.0).

Two-way paper chromatography gets around the problem of separating out substances which have very similar R_f values (Figure 29.2e.). A single spot of mixture placed towards one end of the base line. It is stood in a solvent as before and left until the solvent front gets close to the top of the paper. Once dried, the paper is rotated through 90°, and developed again in a different solvent. The spots will separate as the compounds will interact differently with the new mobile phase.

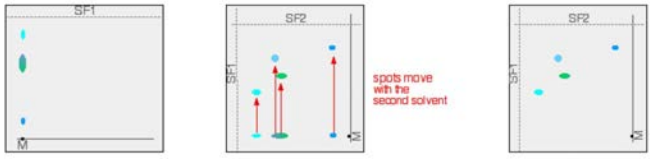


Figure 29.2e. Two-way paper chromatography. a) single dot of sample is placed at M and run with first solvent. b) after drying, the paper is rotated and run with second solvent. c) End result showing the separated sample (credit: *Chromatography* , CC BY-NC 4.0).

Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is done exactly as it says – using a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The silica gel (or the alumina) is the stationary phase. The stationary phase for thin layer chromatography also often contains a substance which fluoresces in UV light. The mobile phase is a suitable liquid solvent or mixture of solvents.

Thin layer chromatography is very similar to paper chromatography in how it is setup, run and analyzed (Figure 29.2f.). Pencil is used to draw the starting line (not ink). The sample spot is allowed to dry before

placing in the beaker. The beaker is covered to ensure the solvent vapour fills the beaker and doesn't escape (especially important when using highly volatile solvents) allowing the solvent to travel up the plate and not evaporate. The solvent level is below that of the sample spot.

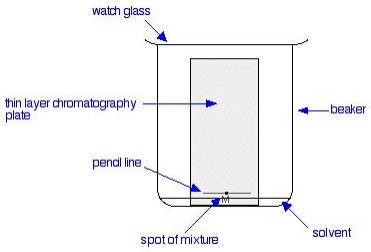


Figure 29.2f. Setup of TLC (thin layer chromatography) (credit: *Chromatography*, CC BY-NC 4.0).

As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates and the mixture is separated into different coloured spots. Figure 29.2g. shows the plate after the solvent has moved about halfway up it. The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the dye components for this particular combination of solvent and stationary phase.

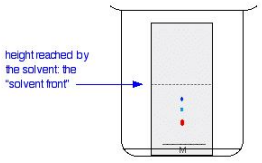
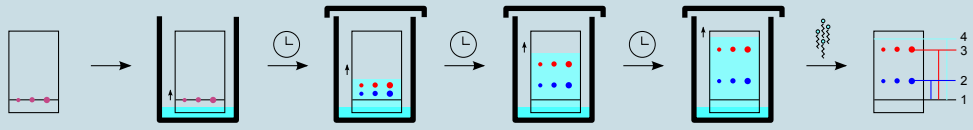


Figure 29.2g. TLC experiment about halfway through mobile phase movement (credit: *Chromatography*, CC BY-NC 4.0).

The resulting TLC separation in then analyzed. If you wanted to know is how many different components made up the mixture, it could be determined visually. However, R_f measurements are often taken from the plate in order to help identify the compounds present. These measurements, as with paper chromatography, are the distance traveled by individual spots divided by the distance traveled by the solvent. When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

Exercise 29.2a

In this TLC experiment, three dots of purple ink are placed on the base line (1). The TLC is exposed to solvent and the solvent front is allowed to reach the top of the plate (line 4). After drying, the distance the red (3) and blue (2) dots travelled are recorded. Calculate the R_f value for each coloured dot using these values. (1) is at 0.0 cm, (2) is at 1.9 cm. (3) is at 4.7 cm and (4) is 6.1 cm.



(Credit: Image by Shakiestone, edited by Quantockgoblin, Public Domain)

Check Your Answer:[1]

If you could repeat this experiment under exactly the same conditions, then the R_f values for each component would always be the same. However, if anything changes (the temperature, the exact composition of the solvent, and so on), that is no longer true.

If the components of the mixture being separated are not visible (colourless), they can be spotted using fluorescence (hence the UV fluorescent compound in the TLC coating). The TLC plate will glow under UV light and the glow is masked at the position where the spots are. The spots show up as darker patches (Figure 29.2h.). Using pencil while under UV light, these spots can be marked on the plate for analysis. Exposing the TLC plate to chemicals may also allow the spots to be seen (see Figure 29.2d.).

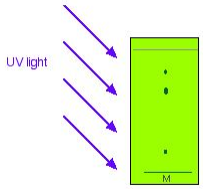


Figure 29.2h. TLC plate glowing under UV light with the separate components as dull dots (credit: *Chromatography*, CC BY-NC 4.0).

The TLC process relies on physical differences between the stationary and mobile phases and the components of the sample being tested. As the solvent (mobile phase) begins to soak up the TLC plate (stationary phase),

it first dissolves the compounds in the spot on the base line. The compounds present will then tend to get carried up the chromatography plate as the solvent continues to move upwards. How fast the compounds get carried up the plate depends on two things:

- How soluble the compound is in the solvent. This will depend on how much attraction there is between the molecules of the compound and those of the solvent.
- How much the compound sticks (adsorb) to the stationary phase – the silica gel or alumina. This will depend on how much attraction there is between the molecules of the compound and the silica gel.

Adsorption isn't permanent – there is a constant movement of a molecule between being adsorbed onto the silica gel surface and going back into solution in the solvent. Obviously, the compound can only travel up the plate during the time that it is dissolved in the solvent. While it is adsorbed on the silica gel, it is temporarily stopped – the solvent is moving on without it. That means that the more strongly a compound is adsorbed, the less distance it can travel up the plate.

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- "A. Introducing Chromatography: Thin Layer Chromatography" and "E. Paper Chromatography" by Jim Clark (Chemguide.co.uk (http://www.chemguide.co.uk)) In *Chromatography*, CC BY-NC 4.0 . / Content has been reorganized and combined to improve student understanding.

Notes

1. Red $R_f = 0.77$, Blue $R_f = 0.31$

29.3 CHROMATOGRAPHIC COLUMNS

Learning Objectives

By the end of this section, you will be able to:

- Describe the purpose and procedure of column chromatography.

The same principles used in thin layer chromatography and paper chromatography can be applied on a larger scale to separate mixtures in column chromatography. Column chromatography is often used to purify compounds made in the lab.

In thin layer chromatography, the stationary phase is a thin layer of silica gel or alumina on a glass, metal or plastic plate. Column chromatography works on a much larger scale by packing the same materials into a vertical glass column (Figure 29.3a.). Various sizes of chromatography columns are used.

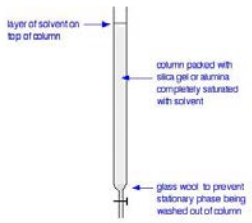

Figure 29.3a. Chromatography column (credit: *Chromatography*, CC BY-NC 4.0).

Separating Mixtures with Columns

To separate a green mixture of two-coloured compounds, one yellow, one blue, it starts with a concentrated solution of the mixture preferably in the solvent used in the column. First, open the tap to allow the solvent already in the column to drain so that it is level with the top of the packing material, and then add the solution carefully to the top of the column. Then, open the tap again so that the coloured mixture is all absorbed into the top of the packing material, so that it might look like Figure 29.3b.

Figure 29.3b. Start of a chromatography column with a green mixture (credit: *Chromatography*, CC BY-NC 4.0).

Next, add fresh solvent to the top of the column, trying to disturb the packing material as little as possible. Then, open the tap so that the solvent can flow down through the column, collecting it in a beaker or flask at the bottom. As the solvent runs through, keep adding fresh solvent to the top so that the column never dries out. Figure 29.3c. shows what might happen over time.

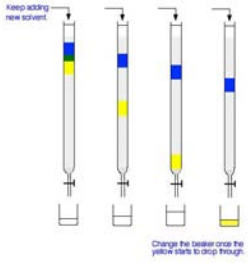

Figure 29.3c. Evolution of green mixture separation over time of adding more solvent (credit: *Chromatography*, CC BY-NC 4.0).

In Figure 29.3c., the blue compound is moving slower than the yellow compound through the column. This means, in this setup, the blue compound is more polar than the yellow one. It must adsorb more strongly to the silica gel or alumina than the yellow one. The less polar yellow one spends more of its time in the solvent and therefore washes through the column much faster. The process of washing a compound through a column using a solvent is known as elution. The solvent is sometimes known as the eluent.

To collect the blue compound, continuing with the same solvent is going to take a lot of time and solvent because it is travelling so slowly. However, there is no reason why the solvent can't be change during elution. By replacing the solvent with a more polar one once the yellow has all been collected, will speed the blue compound through the column. With a more polar solvent, the blue compound spends more time in solution, and so moves faster. By using two different solvents, decent separation of the components is possible as well as keeping waste and time to a minimum.

TLC can be combined with column chromatography if the mixture is colourless by collecting the output in separate containers and testing each with a TLC process.

Instruments that use Chromatographic Columns

Chromatographic columns are part of the instrumentation that is used in chromatography. Several chromatographic methods that use columns are gas chromatography (GC), liquid chromatography (LC), Ion exchange chromatography (IEC), and size exclusion chromatography (SEC). The basic principles of chromatography can be applied to all five methods.

In gas chromatography (GC), the mobile phase is a gas. Gas chromatographic columns are usually between 1 and 100 meters long. The liquid stationary phase is bonded or adsorbed onto the surface of an open tubular (capillary) column, or onto a packed solid support inside the column. Matching the polarities of the analyte and stationary phase is not an exact science. The two should have similar polarities. The thickness of the stationary phase ranges between 0.1 and 8 μm. The thicker the layer the more volatile the analyte can be.

High performance liquid chromatography (HPLC) is a type of liquid chromatography that uses a liquid mobile phase. The same basic principles from gas chromatography are applied to liquid chromatography. There are three basic types of liquid chromatographic columns: liquid-liquid, liquid-solid, and ion-exchange. Liquid-liquid chromatographic columns have the liquid stationary phase bonded or absorbed to the surface of the column, or packed material. liquid-liquid chromatographic columns are not as popular because they have limited stability, and they are inconvenient. Partitioning occurs between the two different liquids of the mobile and stationary phases. In liquid-solid chromatographic columns the stationary phase is a solid and the analyte absorbs onto the stationary phase which separates the components of the mixture. In ion-exchange chromatographic columns the stationary phase is an ion-exchange resin and partitioning occurs with ion exchanges that occur between the analyte and stationary phase. The most common HPLC columns are made from stainless steel, but they can be also made out of thick glass, polymers, a combination of stainless steel and glass, or a combination of stainless steel and polymers. Typical HPLC analytical columns are between 3 and 25 cm long and have a diameter of 1 to 5 mm. The columns are usually straight unlike GC columns. Particles that pack the columns have a typical diameter between 3 to 5 μm. Liquid chromatographic columns will increase in efficiency when the diameter of the packed particles inside the column decreases.

Ion exchange chromatographic (IEC) columns are used to separate ions and molecules that can be easily ionized. Separation of the ions depends on the ion's affinity for the stationary phase, which creates an ion exchange system. The electrostatic interactions between the analytes, mobile phase, and the stationary phase, contribute to the separation of ions in the sample. Only positively or negatively charged complexes can interact with their respective cation or anion exchangers. Common packing materials for ion exchange columns are amines, sulfonic acid, diatomaceous earth, styrene-divinylbenzene, and cross-linked polystyrene resins.

Size exclusion chromatographic (SEC) columns separate molecules based upon their size, not molecular

weight. A common packing material for these columns is molecular sieves. The molecular sieves have pores that small molecules can go into, but large molecules cannot (Figure 29.3d.). This allows the larger molecules to pass through the column faster than the smaller ones. Other packing materials for size exclusion chromatographic columns are polysaccharides and other polymers, and silica. The pore size for size exclusion separations varies between 4 and 200 nm.

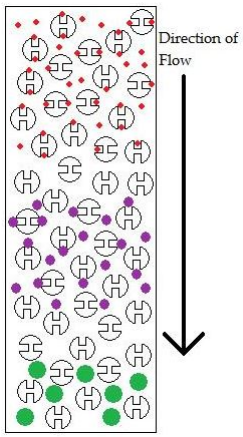

Figure 29.3d. Schematic of a size exclusion column. The larger particles will elute first because they are too big to fit inside the pores. The smallest particles will elute last because they fit very well inside the pores (credit: *Chromatography*, CC BY-NC-SA 4.0).

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29.4 CHROMATOGRAPHY TECHNOLOGY

Learning Objectives

By the end of this section, you will be able to:

- Explain the purpose and general process of gas chromatography.
- Explain the purpose and general process of high-performance liquid chromatography.

Gas Chromatography (GC)

Gas chromatography is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase. The mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column. The stationary phase is either a solid adsorbant, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC).

In early 1900s, Gas chromatography (GC) was discovered by Mikhail Semenovich Tsvett as a separation technique to separate compounds. Among the various types of gas chromatography, gas-liquid chromatography is the method most commonly used to separate organic compounds. The combination of gas chromatography and mass spectrometry is an invaluable tool in the identification of molecules. A typical gas chromatograph consists of an injection port, a column, carrier gas flow control equipment, ovens and heaters for maintaining temperatures of the injection port and the column, an integrator chart recorder and a detector (Figure 29.4a.). Often a mass spectrometer is used as the detector.

To separate the compounds in gas-liquid chromatography, a solution sample that contains organic compounds of interest is injected into the sample port where it will be vaporized. The vaporized samples that are injected are then carried by an inert gas, which is often used by helium or nitrogen. This inert gas goes through a glass column packed with silica that is coated with a liquid. One of three things might happen to a particular molecule in the mixture injected into the column:

- It may condense on the stationary phase.
- It may dissolve in the liquid on the surface of the stationary phase.

- It may remain in the gas phase.

None of these things is necessarily permanent. A compound with a boiling point higher than the temperature of the column will obviously tend to condense at the start of the column. However, some of it will evaporate again in the same way that water evaporates on a warm day – even though the temperature is well below 100°C. The chances are that it will then condense again a little further along the column. Similarly, some molecules may dissolve in the liquid stationary phase. Some compounds will be more soluble in the liquid than others. The more soluble ones will spend more of their time absorbed into the stationary phase; the less soluble ones will spend more of their time in the gas. Any molecule in the substance spends some of its time dissolved in the liquid and some of its time carried along with the gas.

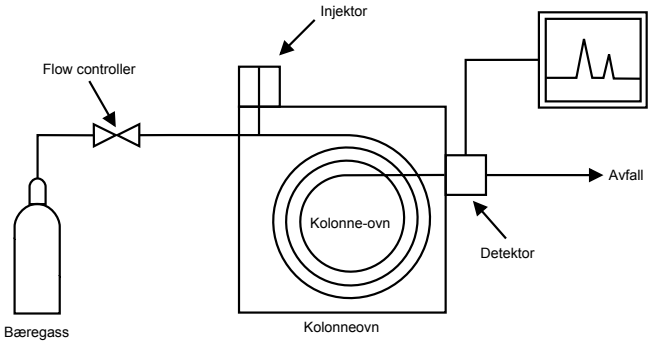

Figure 29.4a. Schematic of gas chromatograph. (Credit: Image by Offnfopt, PD)

The time taken for a particular compound to travel through the column to the detector is known as its retention time. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound. Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

- the boiling point of the compound. A compound which boils at a temperature higher than the column temperature is going to spend nearly all of its time condensed as a liquid at the beginning of the column. So high boiling point means a long retention time.
- the solubility in the liquid phase. The more soluble a compound is in the liquid phase, the less time it will spend being carried along by the gas. High solubility in the liquid phase means a high retention time.
- the temperature of the column. A higher temperature will tend to excite molecules into the gas phase – either because they evaporate more readily, or because they are so energetic that the attractions of the liquid no longer hold them. A high column temperature shortens retention times for everything in the column.

The output from the GC will be recorded as a series of peaks – each one representing a compound in the

mixture passing through the detector. With caution, the retention times can be used to help to identify the compounds present. The peaks can also be used as a way of measuring the relative quantities of the compounds present.

Gas chromatography is a physical separation method in where volatile mixtures are separated (Figure 29.4b.). It can be used in many different fields such as pharmaceuticals, cosmetics and even environmental toxins. Since the samples have to be volatile, human breathe, blood, saliva and other secretions containing large amounts of organic volatiles can be easily analyzed using GC. Knowing the amount of which compound is in a given sample gives a huge advantage in studying the effects of human health and of the environment as well.

Air samples can be analyzed using GC. Most of the time, air quality control units use GC coupled with FID (flame ionization detector) in order to determine the components of a given air sample. Although other detectors are useful as well, FID is the most appropriate because of its sensitivity and resolution and also because it can detect very small molecules as well. The main disadvantage is that it destroys everything coming out of the column as it detects it. If the product is to be sent to a mass spectrometer, for example, for further analysis, a flame ionization detector cannot be used.

GC/MS (gas chromatography coupled with mass spectrometry) is also another useful method which can determine the components of a given mixture. When the detector is showing a peak, some of what is passing through the detector at that time can be diverted to a mass spectrometer. There it will give a fragmentation pattern which can be compared against a computer database of known patterns. That means that the identity of a huge range of compounds can be found without having to know their retention times. This method be applied to many pharmaceutical applications such as identifying the amount of chemicals in drugs. Moreover, cosmetic manufacturers also use this method to effectively measure how much of each chemical is used for their products.

A simulation of separation of mixture using GC showing the blue component (nonane) eluting first in the graph and the red component (decane) being retained by the stationary phase and eluting later in the graph.

Figure 29.4b. Simulation of separation of mixture using GC. (Credit: Gif by Dvstechnique, PDM)

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is an analytical technique used for the separation of compounds soluble in a particular solvent. Liquid chromatography was initially discovered as an analytical technique in the early twentieth century and was first used as a method of separating coloured compounds. This is where the name chromatography *chroma* means colour, *graphy* means writing, was derived.

A Russian botanist named Mikhail S. Tswett used a rudimentary form of chromatographic separation to purify mixtures of plant pigments into the pure constituents. He separated the pigments based on their interaction with a stationary phase, which is essential to any chromatographic separation. The stationary phase he used was powdered chalk and aluminia, the mobile phase in his separation was the solvent. After the solid stationary phase was packed into a glass column (essentially a long, hollow, glass tube), he poured the mixture of plant pigments and solvent in the top of the column. He then poured additional solvent into the column until the samples were eluted at the bottom of the column. The result of this process was that the plant pigments separated into bands of pure components as they passed through the stationary phase. Modern high performance liquid chromatography or HPLC has its roots in this separation, the first form of liquid chromatography. The chromatographic process has been significantly improved over the last hundred years, yielding greater separation efficiency, versatility and speed.

While all of these basic principles hold true for all chromatographic separations, HPLC was developed as method to solve some of the shortcomings of standard liquid chromatography. Classic liquid chromatography (such as using chromatographic column with gravity) has several severe limitations as a separation method. When the solvent is driven by gravity, the separation is very slow. Instead of a solvent being allowed to drip through a column under gravity, in HPLC, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. Another limiting factor in liquid chromatography was originally the size of the column packing, once columns could be packed with much smaller particles a much greater surface area for interactions between the stationary phase and the molecules flowing past it was created. This allowed a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

Specialized apparatus (Figure 29.4c.) is required for an HPLC separation because of the high pressures and low tolerances under which the separation occurs. If the results are to be reproducible, then the conditions of the separation must also be reproducible. Thus, HPLC equipment must be of high quality; it is therefore expensive.

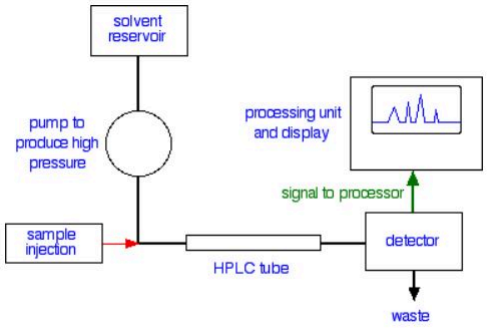

Figure 29.4c. Schematic of HPLC (credit: *Chromatography*, CC BY-NC 4.0).

The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid components whose respective concentrations are varied depending on the composition of the sample. As the solvent is passed through a very narrow bore column, any contaminants could at worst plug the column, or at the very least add variability to the retention times during repeated different trials. Therefore, HPLC solvent must be kept free of dissolved gases, which could come out of solution mid-separation, and particulates. In the HPLC column, the components of the sample separate based on their differing interactions with the column packing (stationary phase). Columns can be packed with solids such as silica or alumina.

Retention time is the amount of time it will take for a compound to travel the length of the HPLC column. The more time a compound spends adsorbed to the stationary phase, the more time the compound will take to travel the length of the column. Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

- the pressure used (because that affects the flow rate of the solvent)
- the nature of the stationary phase (not only what material it is made of, but also particle size)
- the exact composition of the solvent
- the temperature of the column

That means that conditions have to be carefully controlled if you are using retention times as a way of identifying compounds. Whereas the amount of time required for a sample that does not interact with the stationary phase to travel the length of the column is known as the void time. No compound can be eluted in less than the void time.

The output will be recorded as a series of peaks – each one representing a compound in the mixture passing through the detector and absorbing UV light. With caution, the retention times may be used to help to identify the compounds present. The peaks can also be used as a way of measuring the quantities of the compounds present. The resolution of an elution is a quantitative measure of how well two elution peaks can be differentiated in a chromatographic separation (Figure 29.4d.).

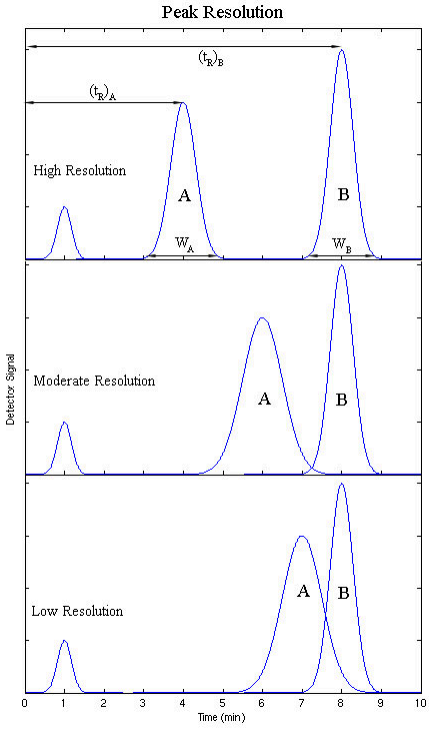

Figure 29.4d. Output of HPLC showing high, moderate and low resolution between peaks A and B (credit: *Chromatography*, CC BY-NC-SA 4.0).

The HPLC detector, located at the end of the column, must register the presence of various components of the sample, but must not detect the solvent. For that reason, there is no universal detector that works for all separations. A common HPLC detector is a UV absorption detector, as most medium to large molecules absorb UV radiation. When the detector is showing a peak, some of what is passing through the detector at that time can be diverted to a mass spectrometer. There it will give a fragmentation pattern which can be compared against a computer database of known patterns. That means that the identity of a huge range of compounds can be found without having to know their retention times. Detectors that measure fluorescence and refractive index are also used for special applications. A relatively new development is the combination of an HPLC separation with an NMR detector. This allows the pure components of the sample to be identified and quantified by nuclear magnetic resonance after having been separated by HPLC, in one integrated process.

HPLC can be used in both qualitative and quantitative applications, that is for both compound identification and quantification. It can be used to effectively separate similar simple and aromatic hydrocarbons, even those that differ only by a single methylene group. It also effectively separates simple amines, sugars, lipids, and even pharmaceutically active compounds. Finally, HPLC is used to separate molecules of biological origin. The determination of caffeine content in coffee products is routinely done by HPLC in commercial applications in order to guarantee purity and quality of ground coffee. HPLC is a useful addition to an analytical arsenal, especially for the separation of a sample before further analysis.

Attribution & References

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29.5 SPECTROSCOPY BASICS

Learning Objectives

By the end of this section, you will be able to:

- Explain the behaviour of matter.
- Describe the electromagnetic spectrum as it applies to spectroscopy.

Spectroscopy is used to determine the structure of a compound. Electromagnetic radiation of a specified range of wavelengths is allowed to pass through a sample containing a compound of interest. The sample molecules absorb energy from some of the wavelengths, and as a result jump from a low energy 'ground state' to some higher energy 'excited state'. Other wavelengths are not absorbed by the sample molecule, so they pass on through. A detector on the other side of the sample records which wavelengths were absorbed, and to what extent they were absorbed.

Behaviour of Matter

Matter can both absorb energy and emit it in the form of electromagnetic radiation such as light. The pattern in which matter absorbs or emits radiation is called its **spectrum**. In the past, and still to this day, studies of the spectrum of a substance have furnished important clues to the structure of matter. At the same time, the spectrum of a substance is often a very useful way of characterizing and hence identifying and analyzing that substance.

Many of the properties of electromagnetic radiation can be explained if light is thought of as periodically varying electric and magnetic fields (electromagnetic waves – Figure 29.5a.). Such waves can be characterized by their frequency υ or their wavelength λ, and their speed of propagation is always $\lambda\upsilon = c = 2.998 \times 10^{8}$ m/s. Some properties of light are more easily explained in terms of particles called photons. The energy of a photon is given by $E = h\upsilon$, where $h = 6.626 \times 10^{-34}$ J s and is called Planck's constant.

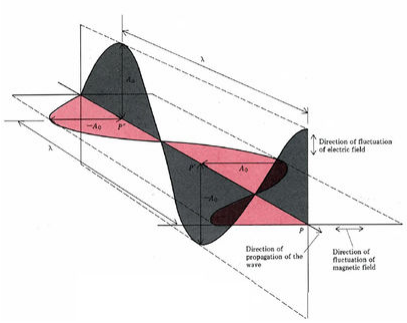

Figure 29.5a. Cross section of electromagnetic wave. (Credit: *ChemPRIME (Moore et al.)*, CC BY-NC-SA 4.0)

When any element is heated to a high temperature or excited in a discharge tube, it gives a line spectrum. Niels Bohr was able to predict the wavelengths of the lines in the spectrum of hydrogen by means of a theory which assigned the single electron to specific energy levels and hence to orbits of specific radius. Absorption of an appropriate quantity of energy can raise the hydrogen atom from a lower to a higher energy level, while emission of electromagnetic radiation corresponds to a change from a higher to a lower energy level. Although Bohr's theory is quantitatively accurate only for hydrogen, his idea of energy levels is useful for all other atoms and even for molecules.

In the case of molecules, energy levels arise because of different speeds and kinds of molecular vibrations and rotations as well as because electrons are moved farther from or closer to positively charged nuclei. In organic compounds some groups of atoms vibrate at much the same frequency no matter what molecule they are in. The energy levels of such vibrations usually differ by roughly the energies of infrared photons, and many organic functional groups can be identified by the characteristic frequencies at which they absorb infrared radiation. When molecules absorb visible or ultraviolet light, band spectra occur (Figure 29.5b.). Some of the energy of each absorbed photon goes to excite an electron, but varying amounts also increase vibrational and rotational energies. Thus, photons are absorbed over a broad range of frequencies and wavelengths.

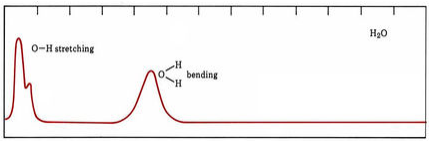

Figure 29.5b. Infrared spectrum of water. (Credit: *ChemPRIME (Moore et al.)*, CC BY-NC-SA 4.0)

Electromagnetic Spectrum

Electromagnetic radiation is composed of electrical and magnetic waves which oscillate on perpendicular planes. Visible light is electromagnetic radiation. So are the gamma rays that are emitted by spent nuclear fuel,

the x-rays that a doctor uses to visualize your bones, the ultraviolet light that causes a painful sunburn when you forget to apply sun block, the infrared light that the army uses in night-vision goggles, the microwaves that you use to heat up your frozen burritos, and the radio-frequency waves that bring music to anybody who is old-fashioned enough to still listen to FM or AM radio.

Just like ocean waves, electromagnetic waves travel in a defined direction. While the speed of ocean waves can vary, however, the speed of electromagnetic waves – commonly referred to as the speed of light – is essentially a constant, approximately 300 million meters per second. This is true whether we are talking about gamma radiation or visible light. Obviously, there is a big difference between these two types of waves – we are surrounded by the latter for more than half of our time on earth, whereas we hopefully never become exposed to the former to any significant degree. The different properties of the various types of electromagnetic radiation are due to differences in their wavelengths (Figure 29.5c.), and the corresponding differences in their energies: shorter wavelengths correspond to higher energy.

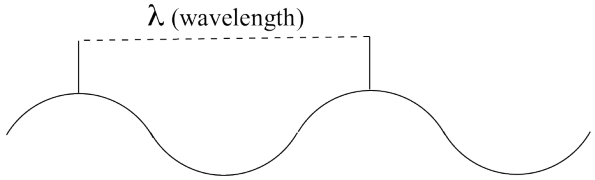

Figure 29.5c. Wavelength of a wave. (Credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)

High-energy radiation (such as gamma- and x-rays) is composed of very short waves – as short as 10^{-16} meter from crest to crest. Longer waves are far less energetic, and thus are less dangerous to living things. Visible light waves are in the range of 400 – 700 nm (nanometers, or 10^{-9} m), while radio waves can be several hundred meters in length.

Because electromagnetic radiation travels at a constant speed, each wavelength corresponds to a given frequency, which is the number of times per second that a crest passes a given point. Longer waves have lower frequencies, and shorter waves have higher frequencies. Frequency is commonly reported in hertz (Hz), meaning 'cycles per second', or 'waves per second'. When talking about electromagnetic waves, we can refer either to wavelength or to frequency – the two values are interconverted using the simple expression:

$$\lambda\nu = c$$

where ν (the Greek letter '*nu*') is frequency in s^{-1}. Visible red light with a wavelength of 700 nm, for example, has a frequency of 4.29×10^{14} Hz, and an energy of 40.9 kcal per mole of photons. The full range of electromagnetic radiation wavelengths is referred to as the **electromagnetic spectrum** (FIgure 29.5d.).

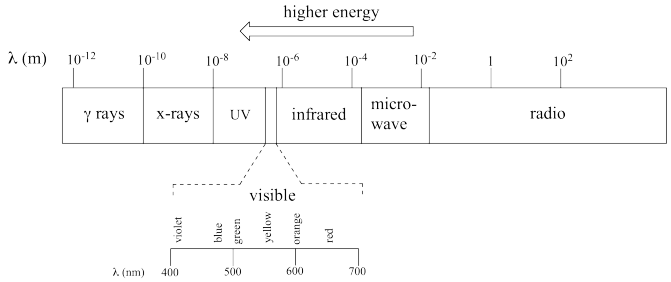

Figure 29.5d. The electromagnetic spectrum. (Credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)

Notice that visible light takes up just a narrow band of the full spectrum. White light from the sun or a light bulb is a mixture of all of the visible wavelengths. You see the visible region of the electromagnetic spectrum divided into its different wavelengths every time you see a rainbow: violet light has the shortest wavelength, and red light has the longest.

Molecular Spectroscopy

In a spectroscopy experiment, electromagnetic radiation of a specified range of wavelengths is allowed to pass through a sample containing a compound of interest. The sample molecules absorb energy from some of the wavelengths, and as a result jump from a low energy 'ground state' to some higher energy 'excited state'. Other wavelengths are not absorbed by the sample molecule, so they pass on through. A detector on the other side of the sample records which wavelengths were absorbed, and to what extent they were absorbed.

Here is the key to molecular spectroscopy: a given molecule will specifically absorb only those wavelengths which have energies that correspond to the energy difference of the transition that is occurring.

Thus, if the transition involves the molecule jumping from ground state A to excited state B, with an energy difference of ΔE, (Figure 29.5e.) the molecule will specifically absorb radiation with wavelength that corresponds to ΔE, while allowing other wavelengths to pass through unabsorbed.

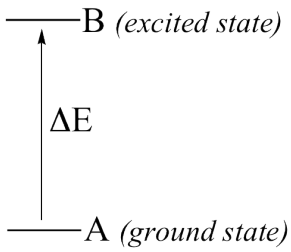

Figure 29.5e. Change in energy required to move molecule from ground state (A) to excited state (B). (Credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)

By observing which wavelengths a molecule absorbs, and to what extent it absorbs them, we can gain information about the nature of the energetic transitions that a molecule is able to undergo, and thus information about its structure.

To get an introduction to infrared spectroscopy (Chapter 29.6) and mass spectrometry (Chapter 29.7), **watch IR Spectroscopy and Mass Spectrometry: Crash Course Organic Chemistry #5 on YouTube (14 min) (https://youtu.be/xMa1BQ8z9C0)**

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Combined and modified to improve student understanding.

29.6 INFRARED (IR) SPECTROSCOPY

Learning Objectives

By the end of this section, you will be able to:

- Describe how molecules vibrate and how an IR spectrum is formed.
- Explain the IR spectrum in terms of general organic functional groups.
- Correlate parts of a molecule's structure and its IR spectrum.

Vibrating Molecules

Photon energies associated with the infrared (from 1 to 15 kcal/mole) (Figure 29.6a.) are not large enough to excite electrons but may induce vibrational excitation of covalently bonded atoms and groups.

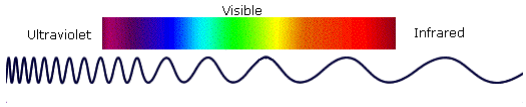


Figure 29.6a. Infrared wavelengths. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

The covalent bonds in molecules are not rigid sticks or rods, such as found in molecular model kits, but are more like stiff springs that can be stretched and bent. We must now recognize that, in addition to the rotation of groups about single bonds, molecules experience a wide variety of vibrational motions, characteristic of their component atoms. Consequently, virtually all organic compounds will absorb infrared radiation that corresponds in energy to these vibrations. Infrared spectrometers permit chemists to obtain absorption spectra of compounds that are a unique reflection of their molecular structure.

At room temperature, organic molecules are always in motion, as their bonds stretch, bend, and twist. These complex vibrations can be broken down mathematically into individual vibrational modes. Vibrational

modes of bonds within molecules are often given descriptive names, such as stretching, bending, scissoring, rocking and twisting (Figure 29.6b.).

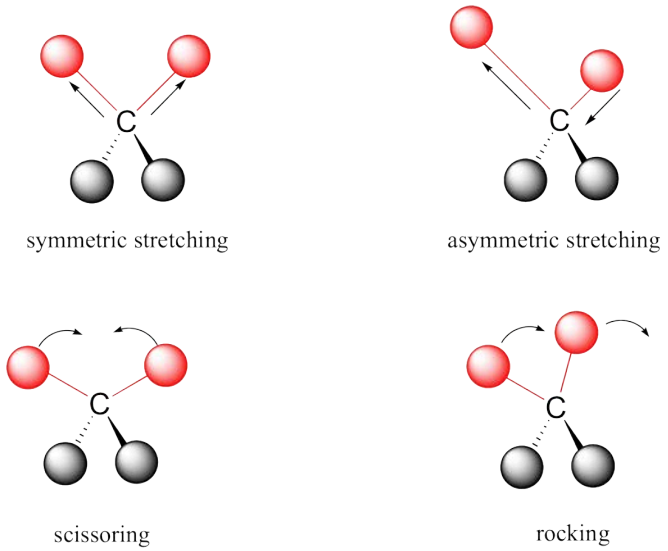


Figure 29.6b. Vibrational modes of stretching, scissoring and rocking.(Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

The energy of molecular vibration is quantized rather than continuous, meaning that a molecule can only stretch and bend at certain 'allowed' frequencies. If a molecule is exposed to electromagnetic radiation that matches the frequency of one of its vibrational modes, it will in most cases absorb energy from the radiation and jump to a higher vibrational energy state – what this means is that the amplitude of the vibration will increase, but the vibrational frequency will remain the same. The difference in energy between the two vibrational states is equal to the energy associated with the wavelength of radiation that was absorbed. It turns out that it is the infrared region of the electromagnetic spectrum which contains frequencies corresponding to the vibrational frequencies of organic bonds.

Infrared (IR) Spectrum

We will use a ketone sample to illustrate this process. The sample is irradiated with infrared light and the carbonyl bond will specifically absorb light with this same frequency. When the carbonyl bond absorbs this energy, it jumps up to an excited vibrational state (Figure 29.6c.). The molecule does not remain in its excited vibrational state for very long, but quickly releases energy to the surrounding environment in form of heat and returns to the ground state.

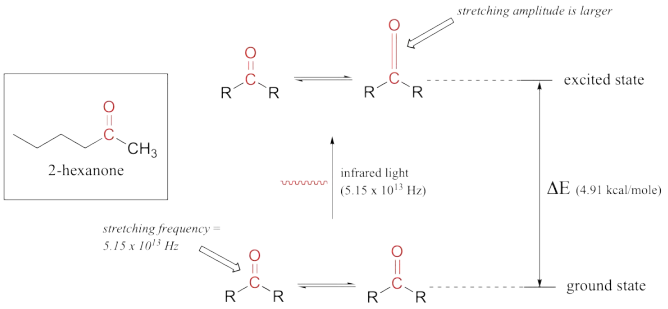


Figure 29.6c. Excitation of carbonyl bond in 2-hexanone. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

With an instrument called an infrared spectrophotometer, we can 'see' this vibrational transition. In the spectrophotometer, infrared light is passed through the sample. Most frequencies pass right through the sample and are recorded by a detector on the other side. Our carbonyl stretching frequency, however, is absorbed by the sample, and so the detector records that the intensity of this frequency, after having passed through the sample, is something less than 100% of its initial intensity. The vibrations of a 2-hexanone molecule are not, of course, limited to the simple stretching of the carbonyl bond. The various carbon-carbon bonds also stretch and bend, as do the carbon-hydrogen bonds, and all of these vibrational modes also absorb different frequencies of infrared light. The IR spectrum for 2-hexanone is shown in Figure 29.6d. The technique is therefore very useful as a means of identifying which functional groups are present in a molecule of interest. If we pass infrared light through an unknown sample and find that it absorbs in the carbonyl frequency range but not in the alkyne range, we can infer that the molecule contains a carbonyl group but not an alkyne.

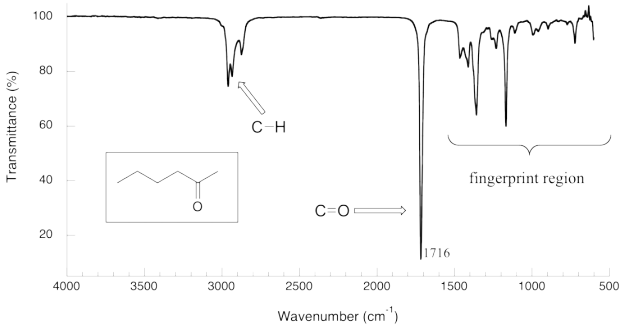


Figure 29.6d. IR spectrum of 2-hexanone. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

The power of infrared spectroscopy arises from the observation that different functional groups have different characteristic absorption frequencies.

Some General Trends:

1. Stretching frequencies are higher than corresponding bending frequencies. (It is easier to bend a bond than to stretch or compress it.)
2. Bonds to hydrogen have higher stretching frequencies than those to heavier atoms.
3. Triple bonds have higher stretching frequencies than corresponding double bonds, which in turn have higher frequencies than single bonds. (Except for bonds to hydrogen).
4. Some bonds do not absorb infrared light at all and will not show in a spectrum.

Infrared spectra may be obtained from samples in all phases (liquid, solid and gaseous). Liquids are usually examined as a thin film sandwiched between two polished salt plates (note that glass absorbs infrared radiation, whereas NaCl is transparent). If solvents are used to dissolve solids, care must be taken to avoid obscuring important spectral regions by solvent absorption. Perchlorinated solvents such as carbon tetrachloride, chloroform and tetrachloroethene are commonly used. Alternatively, solids may either be incorporated in a thin KBr disk, prepared under high pressure, or mixed with a little non-volatile liquid and ground to a paste (or mull) that is smeared between salt plates.

General Functional Group Bands

The general regions of the infrared spectrum in which various kinds of vibrational bands are observed are outlined in Figure 26.9e. and Figure 26.9f. Note that the blue coloured sections above the dashed line refer to stretching vibrations, and the green coloured band below the line encompasses bending vibrations. The complexity of infrared spectra in the 1450 to 600 cm^{-1} region makes it difficult to assign all the absorption bands, and because of the unique patterns found there, it is often called the **fingerprint region**. Absorption bands in the 4000 to 1450 cm^{-1} region are usually due to stretching vibrations of diatomic units, and this is sometimes called the group frequency region.

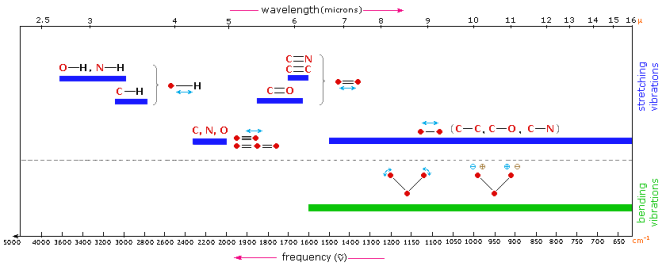
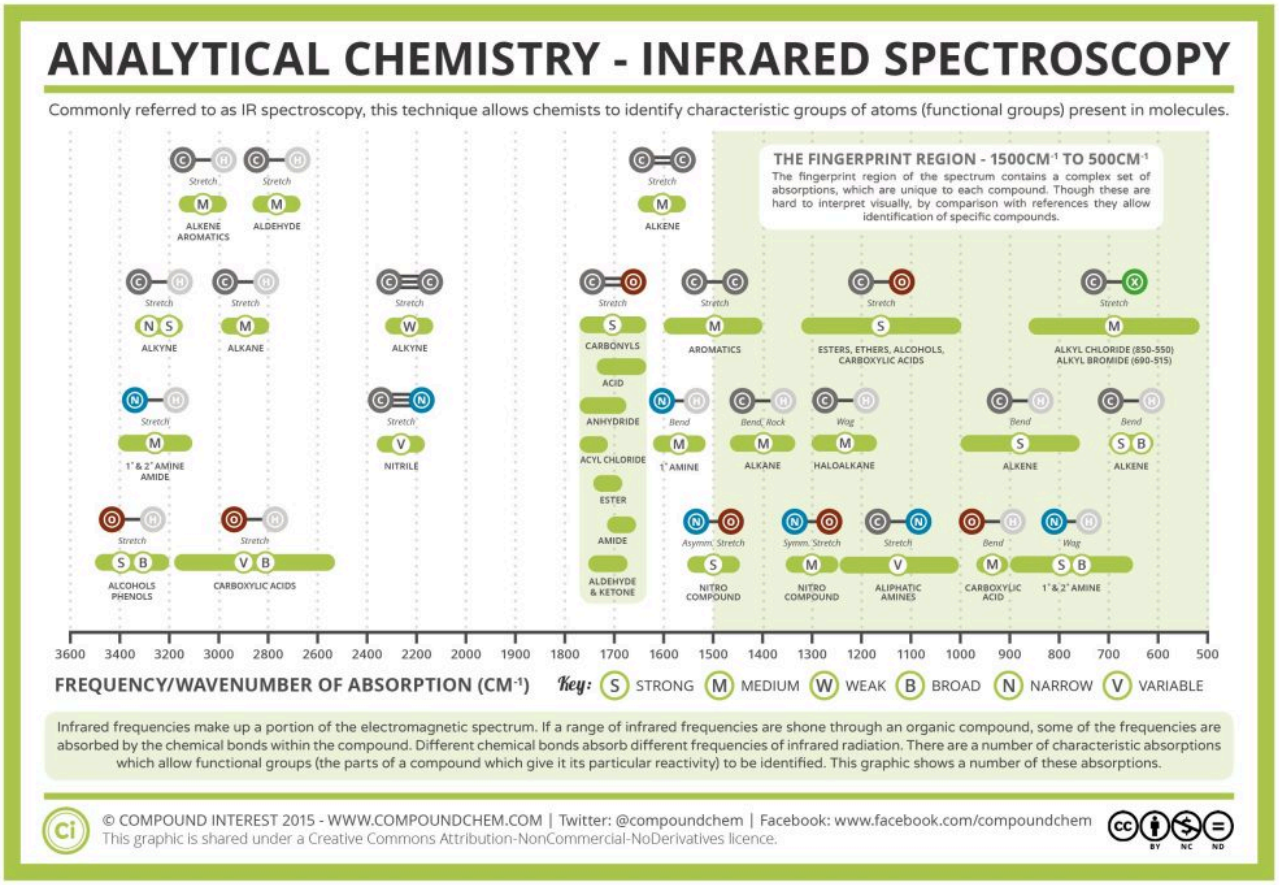


Figure 29.6e. General regions for stretching and bending vibrations of various functional groups. (Credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)



Infographic 29.6a. Infrared spectroscopy common vibrations. Read more about "Analytical Chemistry – Infrared (IR) Spectroscopy (https://www.compoundchem.com/2015/02/05/irspectroscopy/)" by Andy Brunning / Compound Interest, CC BY-NC-ND, or access a text-based summary of infographic 29.6a [New tab].

As you can imagine, obtaining an IR spectrum for a compound will not allow us to figure out the complete structure of even a simple molecule, unless we happen to have a reference spectrum for comparison. In conjunction with other analytical methods, however, IR spectroscopy can prove to be a very valuable tool, given the information it provides about the presence or absence of key functional groups. IR can also be a quick and convenient way for a chemist to check to see if a reaction has proceeded as planned. If we were to run a reaction in which we wished to convert cyclohexanone to cyclohexanol, for example, a quick comparison of the IR spectra of starting compound and product would tell us if we had successfully converted the ketone group to an alcohol.

Example 29.6a

Identify the various vibrations in the infrared spectrum of methanal (formaldehyde), $H_2C{=}O$, given the following data:

- 1750 cm^{-1}
- 2785 cm^{-1}
- 2850 cm^{-1}

Solution

Here is the infrared spectrum of methanal. The fingerprint region from 0 to 1500 cm^{-1} is not typically analyzed.

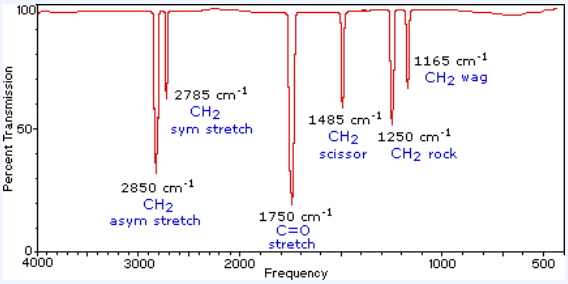


Figure 29.6f. Infrared spectrum of methanal. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

Example 29.6b

Here is the IR spectrum for octanoic acid. Explain the identification of the labelled peaks.

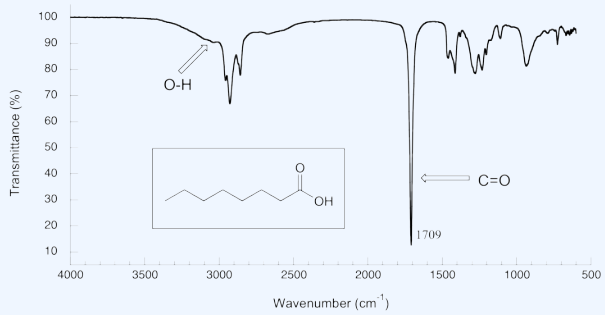


Figure 29.6g. Infrared spectrum of octanoic acid. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

Solution

There is a very broad 'mountain' centered at about 3200 cm^{-1}. This signal is characteristic of the O-H stretching mode of alcohols, and is a dead giveaway for the presence of an alcohol group. The breadth of this signal is a consequence of hydrogen bonding between molecules.

In the spectrum of octanoic acid we see, as expected, the characteristic carbonyl peak, this time at 1709 cm^{-1}. We also see a low, broad absorbance band that looks like an alcohol, except that it is displaced slightly to the right (long-wavelength) side of the spectrum, causing it to overlap to some degree with the C-H region. This is the characteristic carboxylic acid O-H single bond stretching absorbance.

Source: *Map: Organic Chemistry (Wade)* by LibreTexts, licensed under CC BY-NC-SA 4.0

Example 29.6c

Here is the IR spectrum for 1-octene. Explain the identification of the labelled peaks.

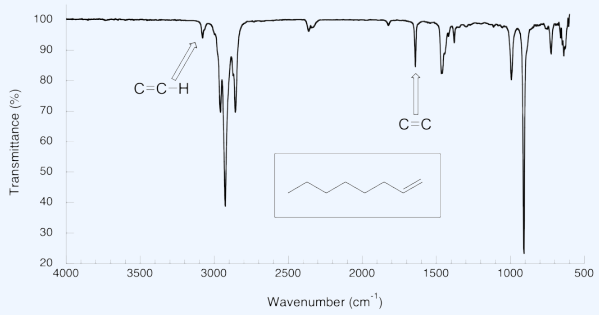


Figure 29.6h. Infrared spectrum of 1-octene. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

Solution

The spectrum for 1-octene shows two peaks that are characteristic of alkenes: the one at 1642cm^{-1} is due to stretching of the carbon-carbon double bond, and the one at 3079 cm^{-1} is due to stretching of the s bond between the alkene carbons and their attached hydrogens.

Alkynes have characteristic IR absorbance peaks in the range of 2100-2250 cm^{-1} due to stretching of the carbon-carbon triple bond, and terminal alkenes can be identified by their absorbance at about 3300 cm^{-1}, due to stretching of the bond between the sp-hybridized carbon and the terminal hydrogen.

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Example 29.6d

Correlate the labelled peaks in these figures to the provided structure and to the data provided in Infographic 29.6a.

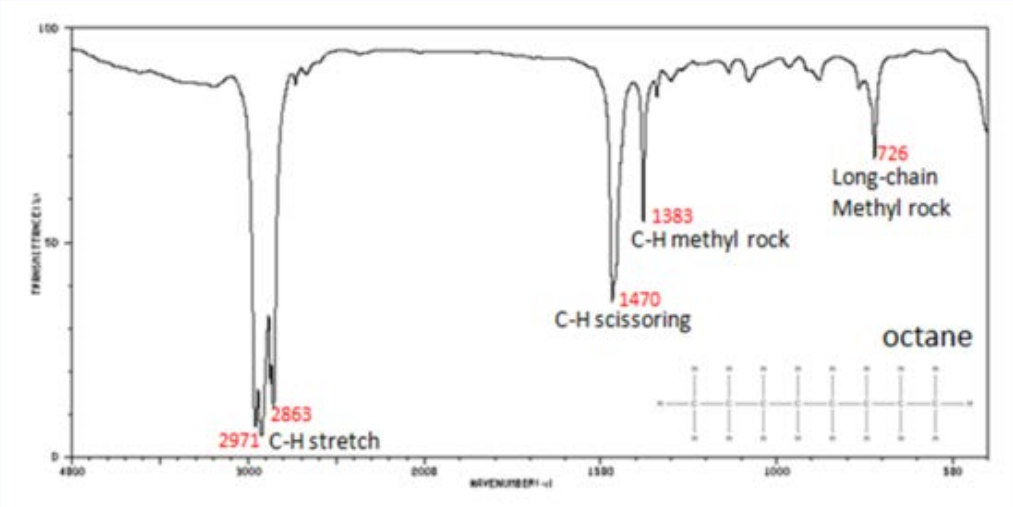

Figure 29.6i. Infrared spectrum of octane (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

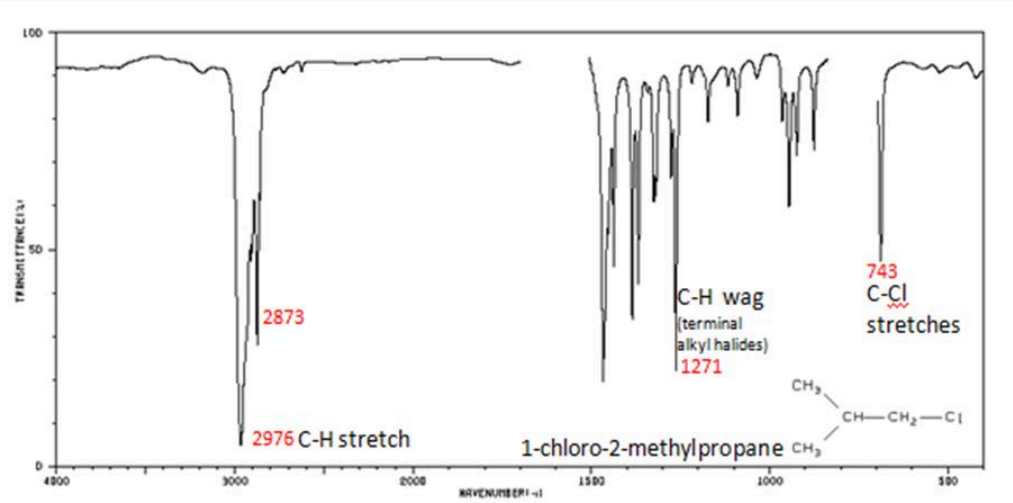

Figure 29.6j. Infrared spectrum of 1-chloro-2-methylpropane (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

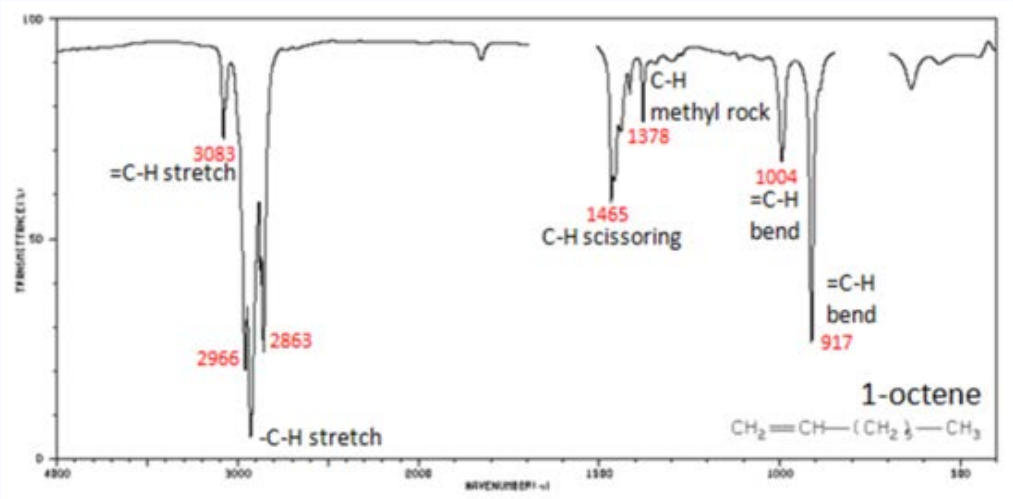

Figure 29.6k. Infrared spectrum of 1-octene (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

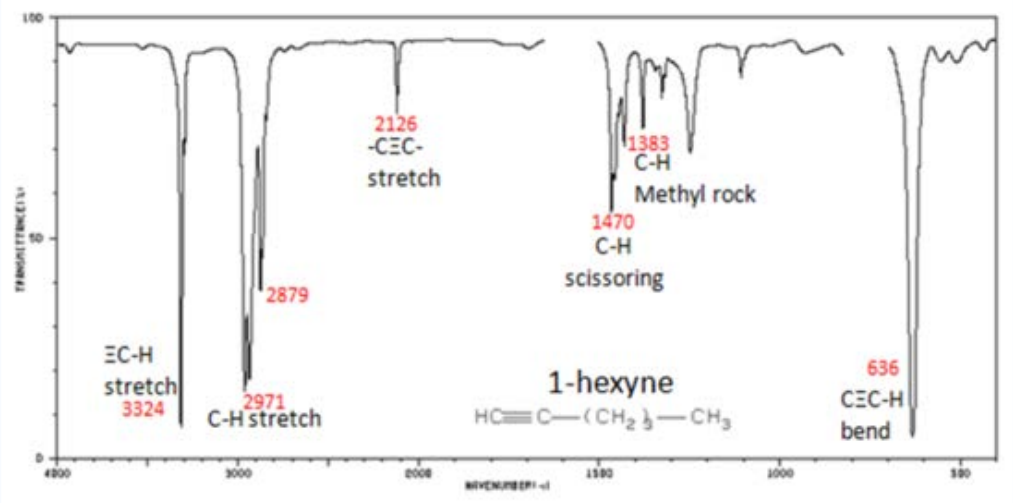

Figure 29.6l. Infrared spectrum of 1-hexyne (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

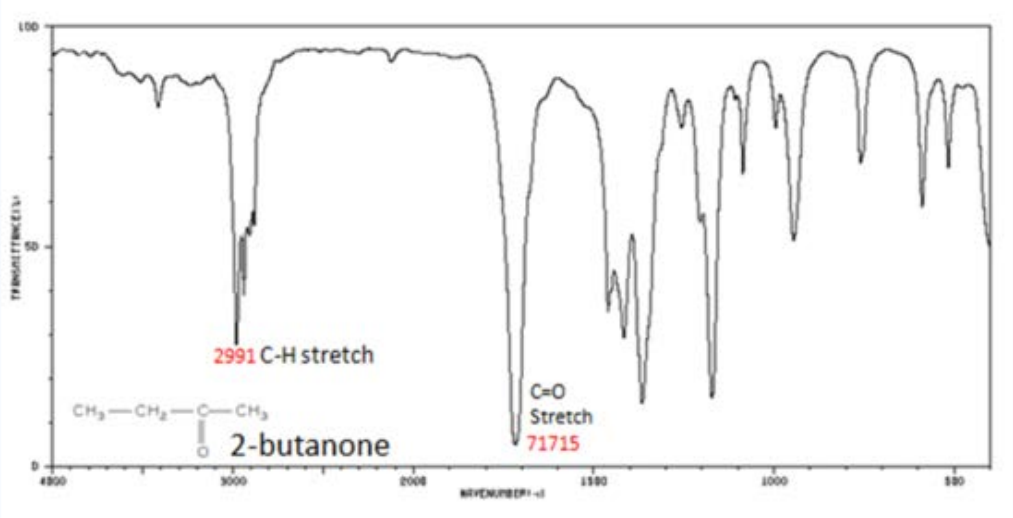

Figure 29.6m. Infrared spectrum of 2-butanone (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

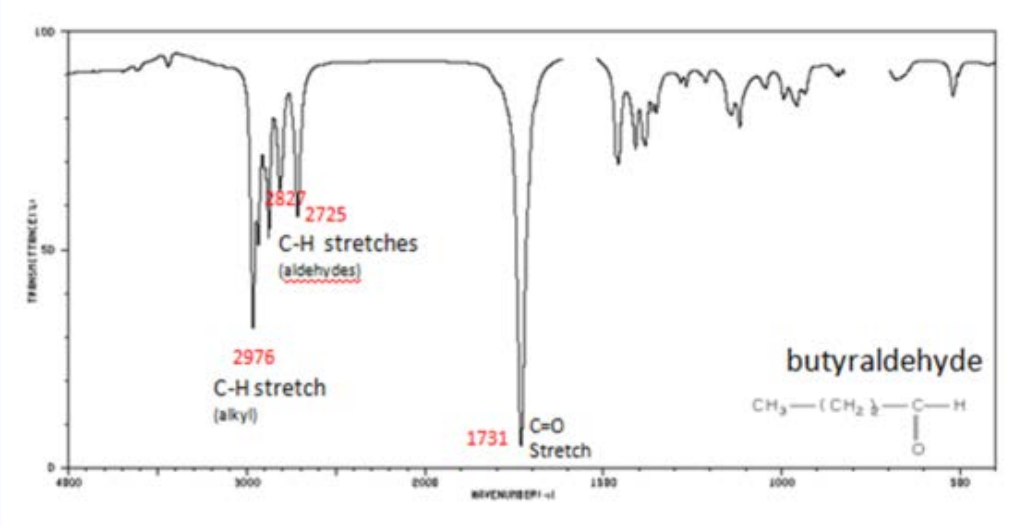

Figure 29.6n. Infrared spectrum of butyraldehyde (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

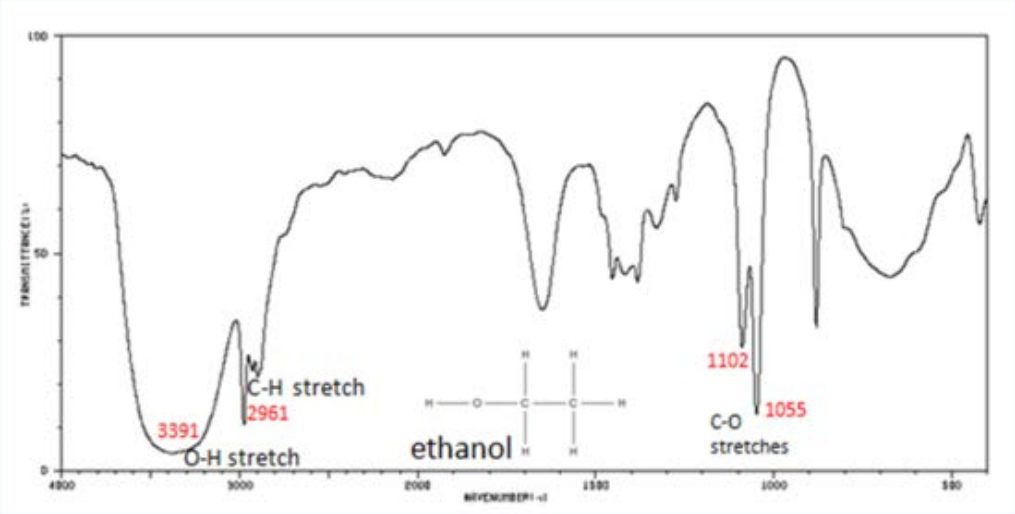

Figure 29.6o. Infrared spectrum of ethanol (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

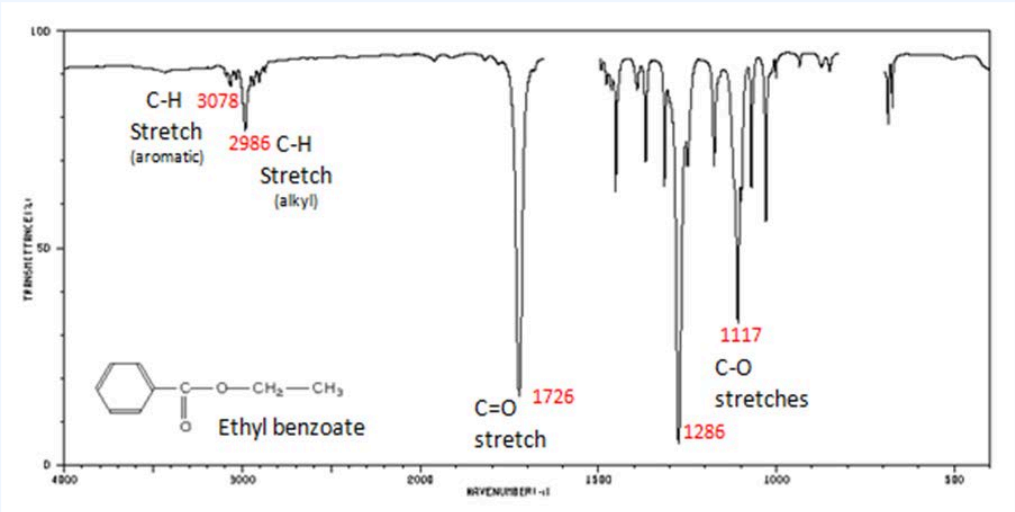

Figure 29.6p. Infrared spectrum of ethyl benzoate (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

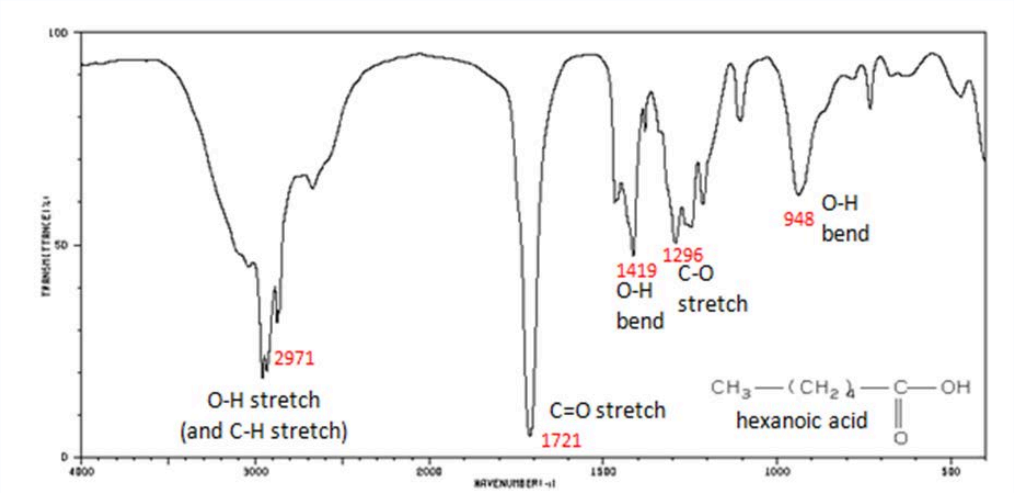

Figure 29.6q. Infrared spectrum of hexanoic acid (credit: *Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

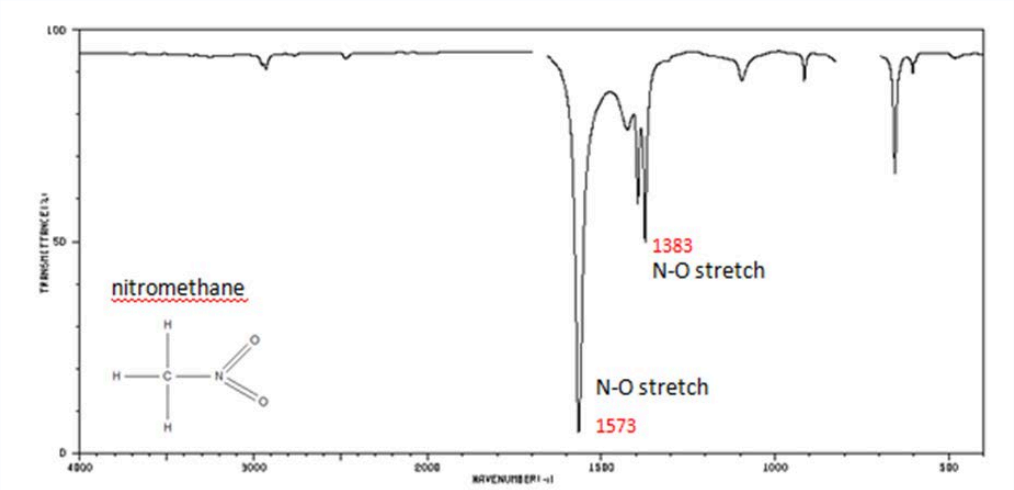

Figure 29.6r. Infrared spectrum of nitromethane (credit: *Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

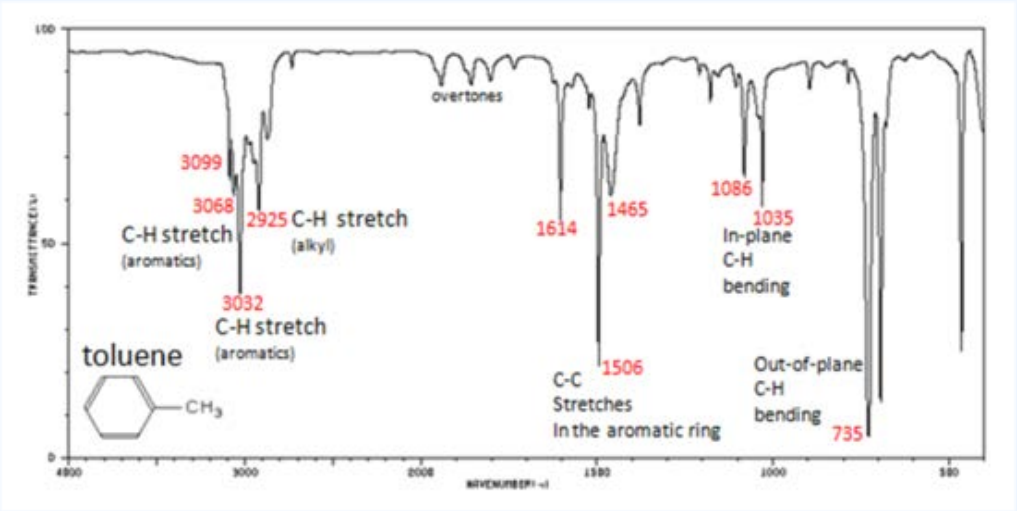

Figure 29.6s. Infrared spectrum of toluene (credit: *Organic Chemistry (Wade)* , CC BY-NC-SA 4.0).

Solutions

Figure 29.6i.: shows the IR spectrum of octane. Since most organic compounds have these features, these C-H vibrations are usually not noted when interpreting a routine IR spectrum. Note that the change in dipole moment with respect to distance for the C-H stretching is greater than that for others shown, which is why the C-H stretch band is the more intense. In alkanes, which have very few bands, each band in the spectrum can be assigned:

- C–H stretch from 3000–2850 cm^{-1}
- C–H bend or scissoring from 1470-1450 cm^{-1}
- C–H rock, methyl from 1370-1350 cm^{-1}
- C–H rock, methyl, seen only in long chain alkanes, from 725-720 cm^{-1}

Figure 29.6j.: Alkyl halides are compounds that have a C–X bond, where X is a halogen: bromine, chlorine, fluorene, or iodine. The alkane components are similar to the answer for Figure 29.6j.

- C–H wag (-CH_2X) from 1300-1150 cm^{-1}
- C–X stretches (general) from 850-515 cm^{-1}
    - C–Cl stretch 850-550 cm^{-1}
    - C–Br stretch 690-515 cm^{-1}

Figure 29.6k.: shows the IR spectrum of 1-octene. As alkanes compounds, these bands are not specific and are generally not noted because they are present in almost all organic molecules. In alkenes compounds, each band in the spectrum can be assigned:

- C=C stretch from 1680-1640 cm^{-1}
- =C–H stretch from 3100-3000 cm^{-1}
- =C–H bend from 1000-650 cm^{-1}

Figure 29.6l.: shows the spectrum of 1-hexyne, a terminal alkyne. In alkynes, each band in the spectrum can be assigned:

- –C≡C– (triple bond) stretch from 2260-2100 cm^{-1}
- –C≡C–H: (triple bond) C–H stretch from 3330-3270 cm^{-1}
- –C≡C–H: (triple bond) C–H bend from 700-610 cm^{-1}

Figure 29.6m.: shows the spectrum of 2-butanone. This is a saturated ketone, and the C=O band appears at 1715 cm^{-1}. The carbonyl stretching vibration band C=O of saturated aliphatic ketones appears:

- C=O stretch – aliphatic ketones 1715 cm^{-1}
- C=O stretch – unsaturated ketones 1685-1666 cm^{-1}

Figure 29.6n.: If a compound is suspected to be an aldehyde, a peak always appears around 2720 cm^{-1} which often appears as a shoulder-type peak just to the right of the alkyl C–H stretches.

- H–C=O stretch 2830-2695 cm^{-1}
- C=O stretch:
    - aliphatic aldehydes 1740-1720 cm^{-1}
    - alpha, beta-unsaturated aldehydes 1710-1685 cm^{-1}

Figure 29.6o.: shows the spectrum of ethanol. Note the very broad, strong band of the O–H stretch. Alcohols have IR absorptions associated with both the O-H and the C-O stretching vibrations.

- O–H stretch, hydrogen bonded 3500-3200 cm^{-1}
- C–O stretch 1260-1050 cm^{-1}

Figure 29.6p.: The carbonyl stretch C=O of esters appears as:

- C=O stretch
    - aliphatic from 1750-1735 cm^{-1}
    - unsaturated from 1730-1715 cm^{-1}
- C–O stretch from 1300-1000 cm^{-1}

Figure 29.6q.: The carbonyl stretch C=O of a carboxylic acid appears as an intense band from 1760-1690

cm^{-1}. The exact position of this broad band depends on whether the carboxylic acid is saturated or unsaturated, dimerized, or has internal hydrogen bonding.

- O–H stretch from 3300-2500 cm^{-1}
- C=O stretch from 1760-1690 cm^{-1}
- C–O stretch from 1320-1210 cm^{-1}
- O–H bend from 1440-1395 and 950-910 cm^{-1}

Figure 29.6r.: In organic nitrogen compounds,

- N–O asymmetric stretch from 1550-1475 cm^{-1}
- N–O symmetric stretch from 1360-1290 cm^{-1}

Figure 29.6s.: In aromatic compounds, each band in the spectrum can be assigned:

- C–H stretch from 3100-3000 cm^{-1}
- overtones, weak, from 2000-1665 cm^{-1}
- C–C stretch (in-ring) from 1600-1585 cm^{-1}
- C–C stretch (in-ring) from 1500-1400 cm^{-1}
- C–H "oop" from 900-675 cm^{-1}

Note that this is at slightly higher frequency than is the –C–H stretch in alkanes. This is a very useful tool for interpreting IR spectra. Only alkenes and aromatics show a C–H stretch slightly higher than 3000 cm^{-1}

Exercise 29.6a

Five infrared spectra are provided for isomers of C_4H_8O. Their structural formulas are also provided in Figure 29.6t. Associate each spectrum (Figure 29.6v through z) with one of the isomers.

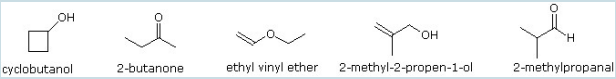

Figure 29.6t. Structure of cyclobutanol, 2-butanone, ethyl vinyl ether, 2-methyl2-propen-1-ol, and 2-methylpropanal. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

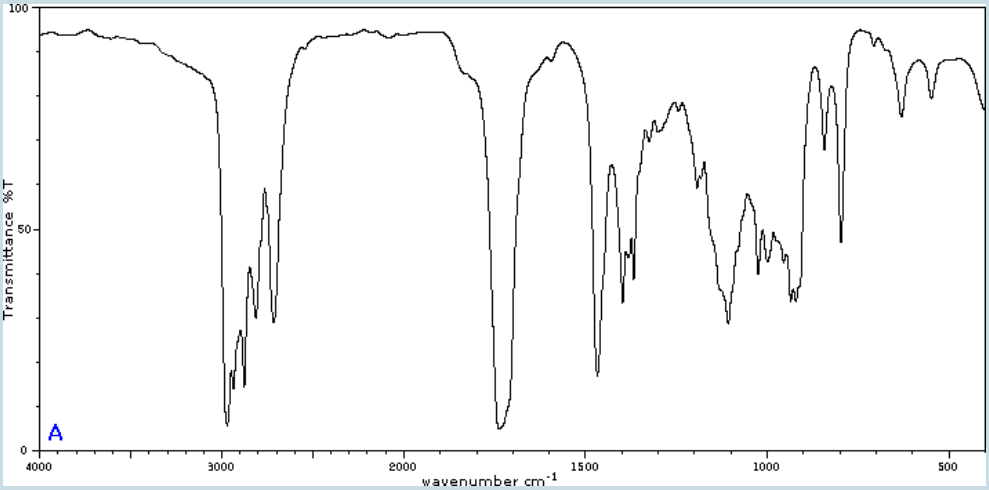

Figure 29.6u. Infrared spectrum of unknown compound A. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

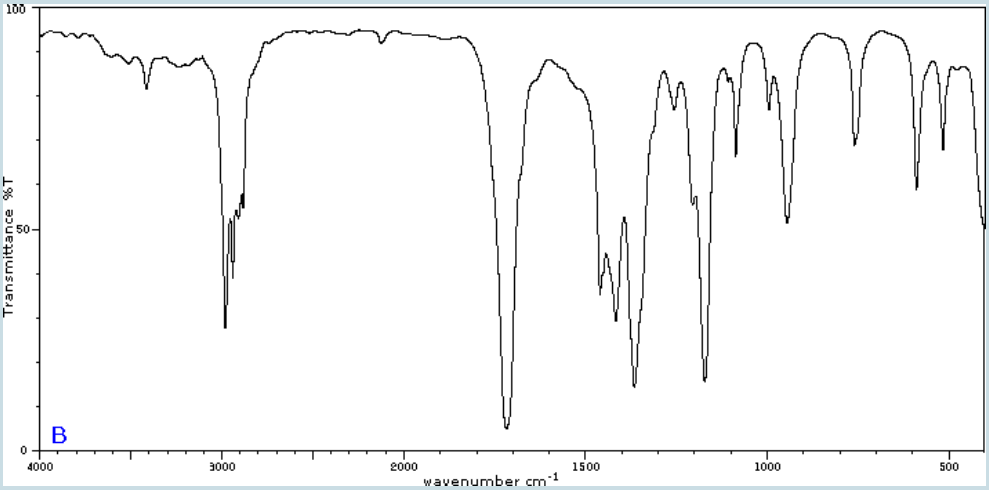

Figure 29.6v. Infrared spectrum of unknown compound B. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

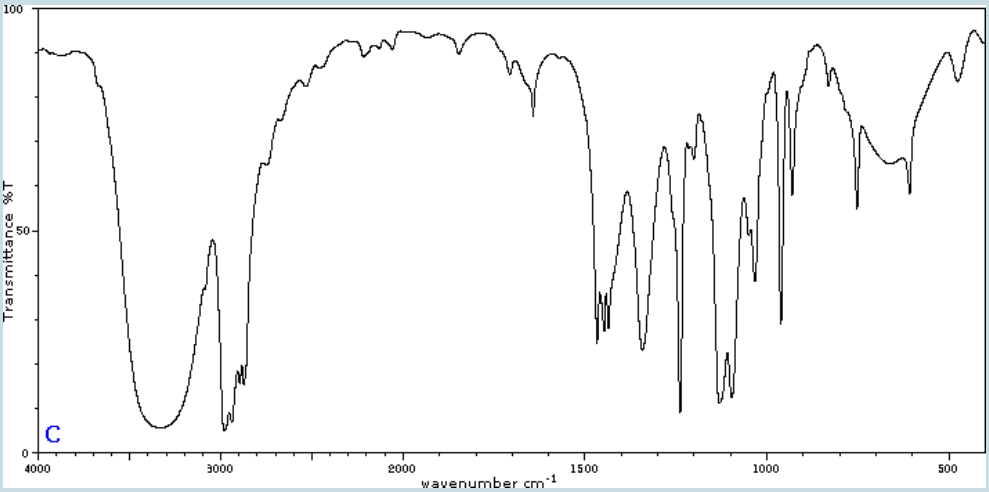

Figure 29.6w. Infrared spectrum of unknown compound C. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

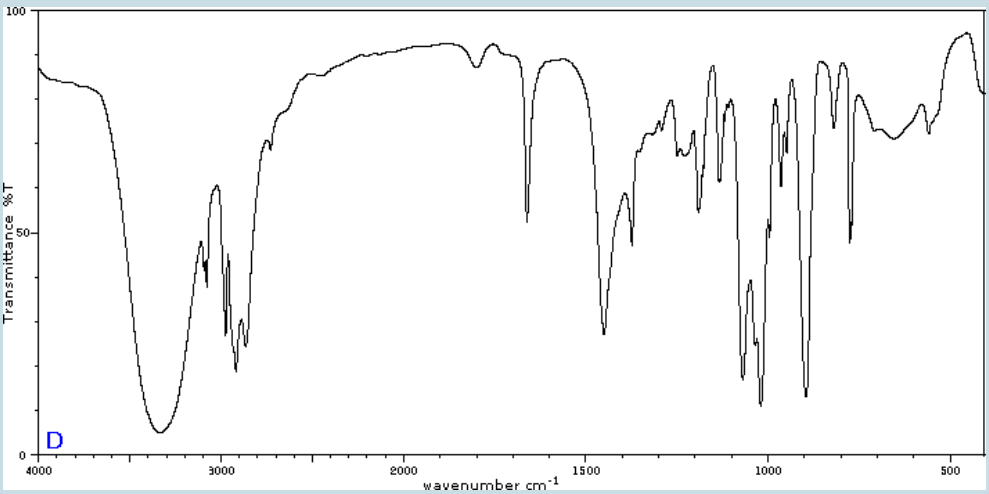

Figure 29.6x. Infrared spectrum of unknown compound D. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

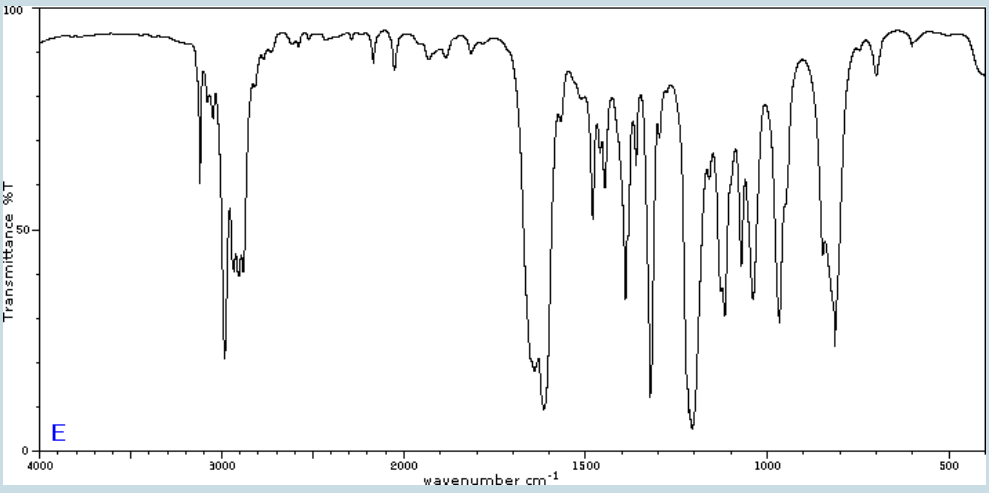

Figure 29.6y. Infrared spectrum of unknown compound E. (Credit: *Map: Organic Chemistry (Wade)* , CC BY-NC-SA 4.0)

Check Your Answers:[1]

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Links to Enhanced Learning

The Chemical Detectives app on Chemical Detectives – Apps on Google Play [New tab] (https://play.google.com/store/apps/details?id=com.chemicaldetectives&pli=1) and Chemical Detectives on the App Store[New tab] (https://apps.apple.com/au/app/chemical-detectives/id741760895) allows users to browse various types of spectra (IR, MS, ^{1}H NMR, ^{13}C NMR, and elemental microanalysis) for simple organic compounds and also complete quizzes about various compounds based on their spectra. Customize of types of functional groups is possible (e.g. only hydrocarbons or only alcohols).

Attribution & References

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  - Prof. Steven Farmer (Sonoma State University (http://www.sonoma.edu))
  - Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

Notes

1. Figure 29.6v: 2-methylpropanal, Figure 29.6w: 2-butanone, Figure 29.6x: cyclobutanol, Figure 29.6y: 2-methyl-2-propen-1-ol, Figure 29.6z: ethyl vinyl ether

29.7 MASS SPECTROMETRY (MS)

Learning Objectives

By the end of this section, you will be able to:

- Identify the essential features of a mass spectrometer.
- Identify peaks in a simple mass spectrum, and explain how they arise.
- Determine the molecular weight of a compound given it's MS spectrum.

Mass spectrometry is an analytic method that employs ionization and mass analysis of compounds in order to determine the mass, formula and structure of the compound being analyzed. A mass analyzer is the component of the mass spectrometer that takes ionized masses and separates them based on charge to mass ratios and outputs them to the detector where they are detected and later converted to a digital output.

Mass spectrometers are large and expensive, and usually operated only by fully trained personnel. Research chemists often rely quite heavily on mass spectra to assist them in the identification of compounds. Note that in most attempts to identify an unknown compound, chemists do not rely exclusively on the results obtained from a single spectroscopic technique. A combination of chemical and physical properties and spectral evidence is usually employed.

The Mass Spectrometer

In order to measure the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be moved about and manipulated by external electric and magnetic fields. The essential functions of a mass spectrometer (Figure 29.7a.) are:

1. **Ionization**: The atom is ionized by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions.

2. **Acceleration:** The ions are accelerated so that they all have the same kinetic energy.
3. **Deflection:** The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected. The amount of deflection also depends on the number of positive charges on the ion – in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.
4. **Detection**: The beam of ions passing through the machine is detected electrically.

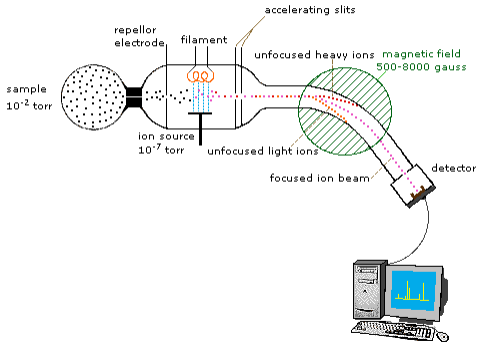


Figure 29.7a. The schematic of a mass spectrometer. (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

Because ions are very reactive and short-lived, their formation and manipulation must be conducted in a vacuum. The heart of the spectrometer is the ion source. Here molecules of the sample (black dots) are bombarded by electrons (light blue lines) issuing from a heated filament. This is called an EI (electron-impact) source. Gases and volatile liquid samples are allowed to leak into the ion source from a reservoir. Non-volatile solids and liquids may be introduced directly. Cations formed by the electron bombardment (red dots) are pushed away by a charged repeller plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments. A perpendicular magnetic field deflects the ion beam in an arc whose radius is inversely proportional to the mass of each ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum).

Formation of Ions and Fragments

When a high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). This leaves behind a molecular ion (coloured red in Figure 29.7b.). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces

(coloured green) and smaller fragment ions (coloured pink and orange). The molecular ion is a radical cation, but the fragment ions may either be radical cations (pink) or carbocations (orange), depending on the nature of the neutral fragment.

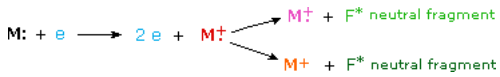


Figure 29.7b. Formation of molecular ions and fragment ions (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

The MS Spectrum

Figure 29.7c. is typical output for an electron-ionization MS experiment of acetone (MS data is derived from the Spectral Database for Organic Compounds, a free, web-based service provided by AIST in Japan).

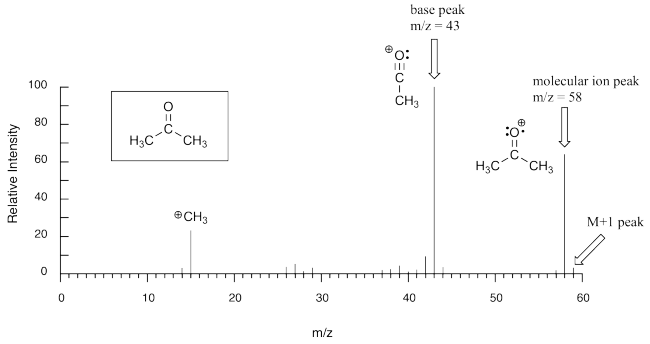


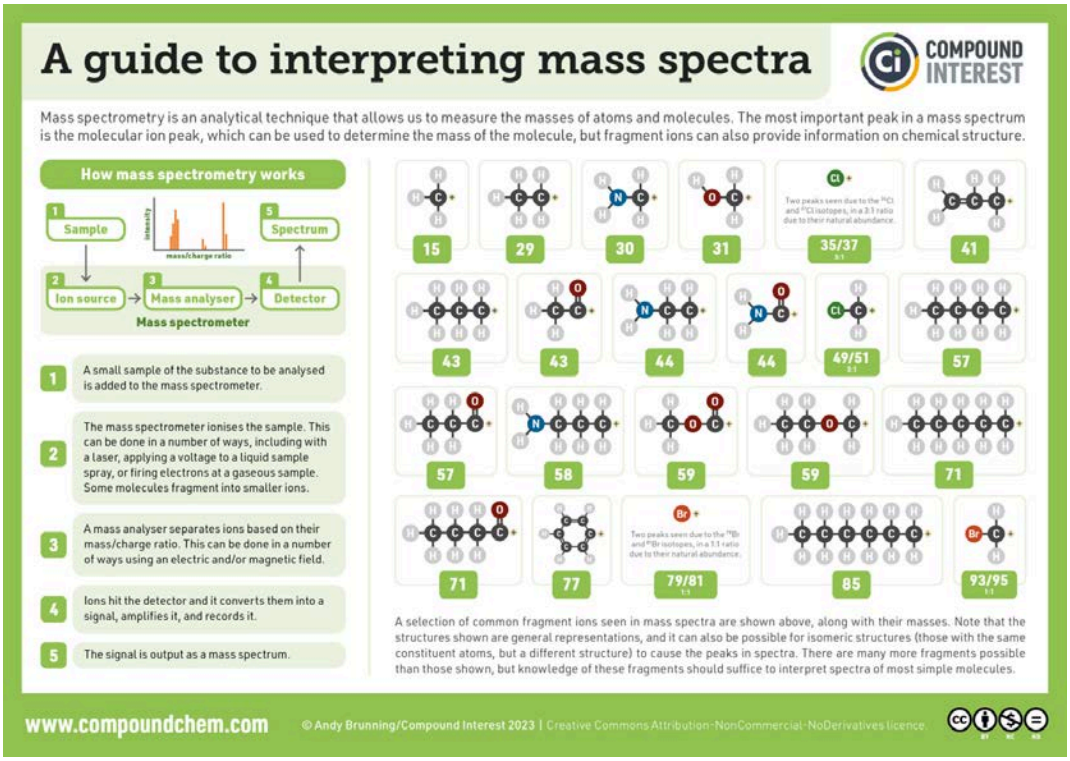
Figure 29.7c. Typical output for an electron-ionization MS experiment examining propanone (or acetone) (MS data for Compound 319 by National Institute of Advanced Industrial Science and Technology via SDBSWeb, Dec. 2, 2016, modified in *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.).

On the horizontal axis is the value for m/z. On the vertical axis is the relative abundance of each ion detected. On this scale, the most abundant ion, called the **base peak**, is set to 100%, and all other peaks are recorded relative to this value.

For acetone, the base peak corresponds to a fragment with m/z = 43. The molecular weight of acetone is 58, so we can identify the peak at m/z = 58 as that corresponding to the **molecular ion peak, or parent peak**.

Notice that there is a small peak at m/z = 59: this is referred to as the M+1 peak. How can there be an ion that has a greater mass than the molecular ion? Simple: a small fraction – about 1.1% – of all carbon atoms in nature are actually the ^{13}C rather than the ^{12}C isotope. The ^{13}C isotope is, of course, heavier than ^{12}C by 1 mass unit. In addition, about 0.015% of all hydrogen atoms are actually deuterium, the ^{2}H isotope. So the M+1 peak represents those few acetone molecules in the sample which contained either a ^{13}C or ^{2}H.

Infographic 29.7a. shows some common anticipated fragmentation ions for interpreting mass spectra.



Infographic 29.7a. A guide to interpreting mass spectrometry. Read more about "Mass spectrometry and a guide to interpreting mass spectra (https://www.compoundchem.com/2015/05/07/mass-spectrometry/)" by Andy Brunning / Compound Interest, CC BY-NC-ND, or access a text-based summary of infographic 29.7a [New tab].

Example 29.7a

Suggest a way of distinguishing between pentan-2-one and pentan-3-one using their mass spectra.

- pentan-2-one – $CH_3COCH_2CH_2CH_3$
- pentan-3-one – $CH_3CH_2COCH_2CH_3$

Solution

Each of these is likely to split to produce ions with a positive charge on the CO group. In the pentan-2-one case, there are two different ions like this:

- $[CH_3CO]^+$
- $[COCH_2CH_2CH_3]^+$

That would give you strong lines at m/z = 43 and 71. With pentan-3-one, you would only get one ion of this kind:

- $[CH_3CH_2CO]^+$

In that case, you would get a strong line at 57. You don't need to worry about the other lines in the spectra – the 43, 57 and 71 lines give you plenty of difference between the two. The 43 and 71 lines are missing from the pentan-3-one spectrum, and the 57 line is missing from the pentan-2-one one.

The two mass spectra look like this:

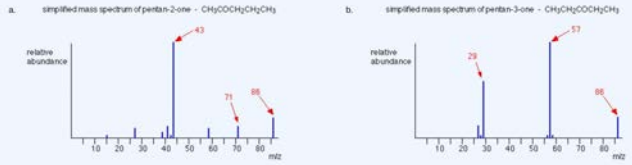


Figure 29.7e. a) Mass spectrum of pentan-2-one and b) Mass spectrum of pentan-3-one (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

As you've seen, the mass spectrum of even very similar organic compounds will be quite different because of the different fragmentation patterns that can occur.

Source: Example 29.7a is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Exercise 29.7a

Caffeine has a mass of 194.19 amu, determined by mass spectrometry, and contains C, N, H, O. What is a molecular formula for this molecule?

Check Your Answer: [1]

Source: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Exercise 29.7b

For each spectrum, justify the molecular weight of the compound and one major peak.

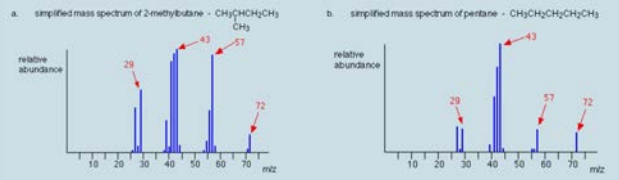


Figure 29.7g. a) Mass spectrum of 2-methylbutane and b) Mass spectrum of pentane (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

Check Your Answers: [2]

Source: Exercise 29.7b written by Samantha Sullivan Sauer with images from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0, based data for Compounds 10633 and 2475 by National Institute of Advanced Industrial Science and Technology via SDBSWeb, Dec. 2, 2016

Links to Enhanced Learning

The Chemical Detectives app on Chemical Detectives on Google Play [New tab] (https://play.google.com/store/apps/details?id=com.chemicaldetectives&pli=1) and Chemical Detectives on the App Store [New tab] (https://apps.apple.com/au/app/chemical-detectives/id741760895) allows users to browse various types of spectra (IR, MS, ^{1}H NMR, ^{13}C NMR, and elemental microanalysis) for simple organic compounds and also complete quizzes about various compounds based on their spectra. Customize of types of functional groups is possible (e.g. only hydrocarbons or only alcohols).

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  - Prof. Steven Farmer (Sonoma State University (http://www.sonoma.edu))
  - Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)
  - Jim Clark (Chemguide.co.uk (http://www.chemguide.co.uk))

Notes

1. $C_8H_{10}N_4O_2$ (C = 12 g/mol × 8 = 96 g/mol; N = 14 g/mol × 4 = 56 g/mol; H = 1 g/mol × 10 = 10 g/mol; O = 16 g/mol x 2 = 32; 96 g/mol + 56 g/mol + 10 g/mol + 32 g/mol = 194 g/mol)

2. a) The molecular weight is 72 g/mol which corresponds to the 72 m/z peak. The three other labelled peaks all correspond to alkane fragments shown in Figure 29.7d. b) The molecular weight is 72 g/mol which corresponds to the 72 m/z peak. The three other labelled peaks all correspond to alkane fragments shown in Figure 29.7d. Notice the similarity with spectrum a) though the abundance of each peak is different due to the structural differences in atom arrangement.

29.8 NUCLEAR MAGNETIC RESONANCE (NMR)

Learning Objectives

By the end of this section, you will be able to:

- Describe how an NMR spectrum is formed.
- Identify the formation and purpose of a chemical shift.

Some types of atomic nuclei act as though they spin on their axis similar to the Earth. Since they are positively charged, they generate an electromagnetic field just as the Earth does. So, in effect, they will act as tiny bar magnetics. Not all nuclei act this way, but fortunately both ^{1}H and ^{13}C do have nuclear spins and will respond to this technique.

NMR Spectrometer

In the absence of an external magnetic field the direction of the spin of the nuclei will be randomly oriented (see Figure 29.8a.). However, when a sample of these nuclei is place in an external magnetic field, the nuclear spins will adopt specific orientations much as a compass needle response to the Earth's magnetic field and aligns with it. Two possible orientations are possible, with the external field (i.e. parallel to and in the same direction as the external field) or against the field (i.e. antiparallel to the external field).

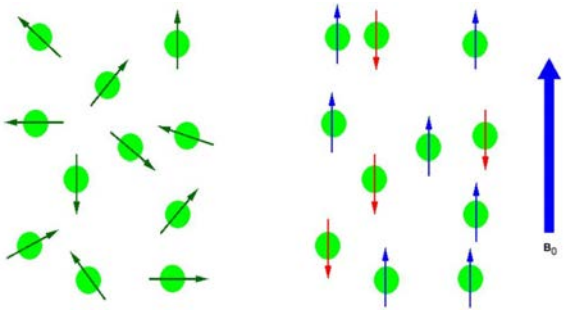

Figure 29.8a. (Left) Random nuclear spin without an external magnetic field. (Right) Ordered nuclear spin in an external magnetic field (credit: *Map: Organic Chemistry (Wade)*, BY-NC-SA 4.0. / Aligned horizontally).

When the same sample is placed within the field of a very strong magnet in an NMR instrument, each hydrogen will assume one of two possible spin states (Figure 29.8b.). In what is referred to as the +½ spin state, the hydrogen's magnetic moment is aligned with the direction of B_0, while in the -½ spin state it is aligned opposed to the direction of B_0.

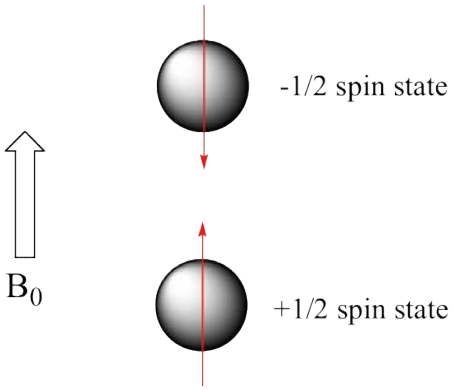

Figure 29.8b. Hydrogen atom alignment within a magnetic field according to spin state (credit: *Map: Organic Chemistry (Wade)*, BY-NC-SA 4.0.).

Because the +½ spin state is slightly lower in energy, in a large population of organic molecules slightly more than half of the hydrogen atoms will occupy this state, while slightly less than half will occupy the –½ state. The difference in energy between the two spin states increases with increasing strength of B_0. With the strong magnetic fields generated by the superconducting magnets used in modern NMR instruments, the resonance frequency for atoms falls within the radio-wave range, anywhere from 100 MHz to 800 MHz depending on the strength of the magnet. The nuclei of atoms aligned with the field will absorb energy and "spin-flip" to align themselves against the field, a higher energy state. When this spin-flip occurs, the nuclei are said to be in "resonance" with the field, hence the name for the technique, Nuclear Magnetic Resonance or NMR.

^{1}H and ^{13}C are not unique in their ability to undergo NMR. All nuclei with an odd number of protons

(^{1}H, ^{2}H, ^{14}N, ^{19}F, ^{31}P ...) or nuclei with an odd number of neutrons (i.e. ^{13}C) show the magnetic properties required for NMR. Only nuclei with even number of both protons and neutrons (^{12}C and ^{16}O) do not have the required magnetic properties.

The basic arrangement of an NMR spectrometer is shown in Figure 29.8c. A sample (in a small glass tube) is placed between the poles of a strong magnetic. A radio frequency generator pulses the sample and excites the nuclei causing a spin-flip. The spin flip is detected by the detector and the signal sent to a computer where it is processed.

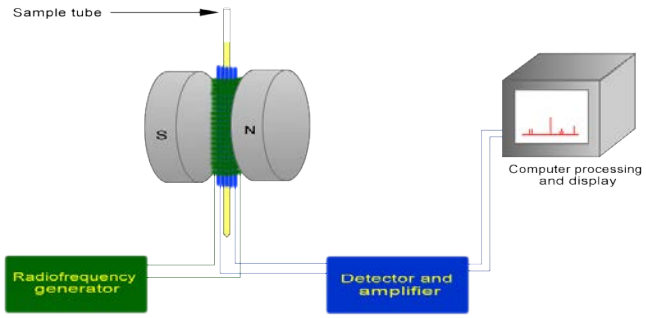

Figure 29.8c. Schematic of NMR spectrometer (credit: *Map: Organic Chemistry (Wade)*, BY-NC-SA 4.0.).

Chemical Shifts

The NMR spectra is displayed as a plot of the applied radio frequency versus the absorption. The applied frequency increases from left to right, thus the left side of the plot is the low field, downfield or deshielded side and the right side of the plot is the high field, upfield or shielded side (see Figure 29.8e.).

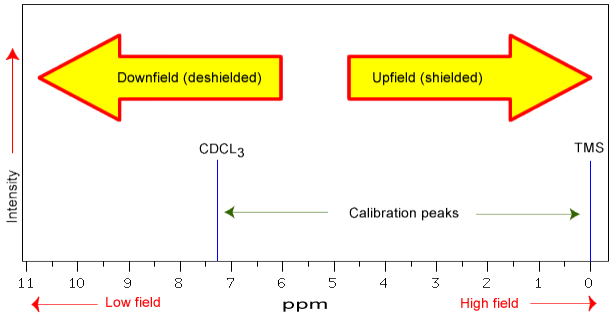

Figure 29.8d. NMR spectrum produced by varying the applied radio frequency. (credit: *Map: Organic Chemistry (Wade)*, BY-NC-SA 4.0.)

The position on the plot at which the nuclei absorbs is called the **chemical shift**. Since this has an arbitrary value a standard reference point must be used. The two most common standards are TMS (tetramethylsilane, $(Si(CH_3)_4)$ which has been assigned a chemical shift of zero, and $CDCl_3$ (deuterochloroform) which has a

chemical shift of 7.26 for $_1$H NMR and 77 for $_{13}$C NMR. The delta (δ) scale is commonly expressed as parts per million (ppm) which is independent of the spectrometer frequency.

The range at which most NMR absorptions occur is quite narrow. Almost all ^{1}H absorptions occur downfield within 10 ppm of TMS. For ^{13}C NMR almost all absorptions occurs within 220 ppm downfield of the C atom in TMS.

Structural features of the molecule will have an effect on the exact magnitude of the magnetic field experienced by a particular nucleus. This means that H atoms which have different chemical environments will have different chemical shifts. This is what makes NMR so useful for structure determination in organic chemistry. There are three main features that will affect the shielding of the nucleus, electronegativity, magnetic anisotropy of π systems and hydrogen bonding.

The electrons that surround the nucleus are in motion so they created their own electromagnetic field. This field opposes the applied magnetic field and so reduces the field experienced by the nucleus. Thus, the electrons are said to shield the nucleus. Since the magnetic field experienced at the nucleus defines the energy difference between spin states it also defines what the chemical shift will be for that nucleus. Electron withdrawing groups can decrease the electron density at the nucleus, deshielding the nucleus and result in a larger chemical shift. These inductive effects are not only felt by the immediately adjacent atoms, but the deshielding can occur further down the chain.

The π electrons in a compound, when placed in a magnetic field, will move and generate their own magnetic field. The new magnetic field will have an effect on the shielding of atoms within the field. The best example of this is benzene (see Figure 29.8e.). This effect is common for any atoms near a π bond.

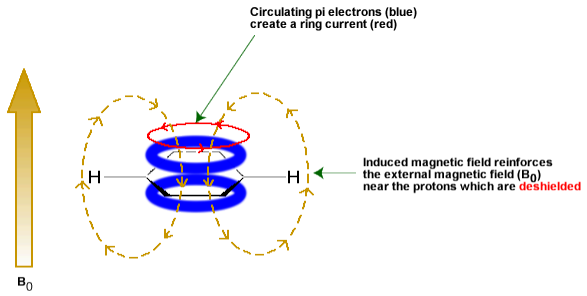

Figure 29.8e. Magnetic anisotropy of π systems (credit: *Map: Organic Chemistry (Wade)*, BY-NC-SA 4.0.)

Protons that are involved in hydrogen bonding (*i.e.*-OH or -NH) are usually observed over a wide range of chemical shifts. This is due to the deshielding that occurs in the hydrogen bond. Since hydrogen bonds are dynamic, constantly forming, breaking and forming again, there will be a wide range of hydrogen bonds strengths and consequently a wide range of deshielding. This as well as solvation effects, acidity, concentration and temperature make it very difficult to predict the chemical shifts for these atoms.

Attribution & References

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- Prof. Steven Farmer (Sonoma State University (http://www.sonoma.edu))
- Dr. Richard Spinney (http://undergrad-ed.chemistry.ohio-state.edu/) (The Ohio State University) (http://chemistry.osu.edu/)

29.9 1H NMR SPECTROSCOPY

Learning Objectives

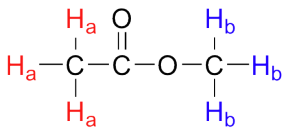
By the end of this section, you will be able to:

- Identify those hydrogen atoms (protons) which are equivalent in a given chemical structure.
- State the approximate chemical shift for various organic functional groups.
- Predict the approximate chemical shifts of each of the protons in an organic compound, given its structure and a table of chemical shift correlations.
- Use an integrated ^{1}H NMR spectrum to determine the ratio of the different types of protons present in an organic compound.
- Explain the spin-spin splitting pattern observed in the ^{1}H NMR spectrum of a simple organic compound.
- Explain what information can be obtained from an integrated ^{1}H NMR spectrum and use this information in the interpretation of such a spectrum.

Building on the principles of NMR explained in the previous section, the interpretation of ^{1}H NMR is the focus of this section. To start, identifying the equivalent hydrogens within an organic molecule is required. In this section, the term proton and hydrogen atom are used interchangeably as a hydrogen atom without its electron is a proton and when bonded hydrogen rarely maintains control of its electron due to its low electronegativity.

Equivalent Hydrogen Atoms

Given that chemically nonequivalent protons (hydrogen atoms) have different resonance frequencies in the same applied magnetic field, we can see how NMR spectroscopy can provide us with useful information about the structure of an organic molecule.



methyl acetate

Figure 29.9a. Structure of methyl acetate (methyl ethanoate) (credit: *Organic Chem: Biological Emphasis vol. 1 (page 242),* CC BY-NC-SA 4.0).

In general, to obtain a NMR spectrum, a sample compound, methyl acetate (methyl ethanoate) (Figure 29.9a.) is placed inside a very strong applied magnetic field (B_0). All of the protons begin to precess: the H_a protons at different frequency than the H_b protons. At first, the magnetic moments of (slightly more than) half of the protons are aligned with B_0, and half are aligned against B_0. Then, the sample is hit with electromagnetic radiation in the radio frequency range. The two specific frequencies which match those of H_a protons and the H_b protons cause those H_a and H_b protons which are aligned with B_0 to 'flip' so that they are now aligned against B_0. In doing so, the protons absorb radiation at the two resonance frequencies. The NMR instrument records which frequencies were absorbed, as well as the intensity of each absorbance.

In most cases, a sample being analyzed by NMR is in solution. If we use a common laboratory solvent (diethyl ether, acetone, dichloromethane, ethanol, water, etc.) to dissolve our NMR sample, however, we run into a problem – there many more solvent protons in solution than there are sample protons, so the signals from the sample protons will be overwhelmed. To get around this problem, we use special NMR solvents in which all protons have been replaced by deuterium. Recall that deuterium is NMR-active, but its resonance frequency is very different from that of protons, and thus it is `invisible` in ^{1}H-NMR. A common NMR solvent is $CDCl_3$ (deuterated trichloromethane/chloroform).

NMR Spectrum

The ^{1}H-NMR spectrum for methyl acetate (methyl ethanoate) is shown in Figure 29.9b. The vertical axis corresponds to intensity of absorbance, the horizontal axis to frequency (typically the vertical axis is not shown in an NMR spectrum).

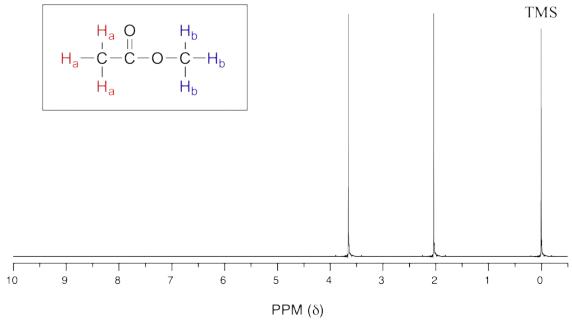
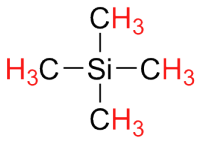


Figure 29.9b. 1H NMR spectrum of methyl ethanoate (credit: *Organic Chem: Biological Emphasis vol. 1 (page 244)*, CC BY-NC-SA 4.0).

There are three absorbance signals: two of these correspond to H_a and H_b, while the peak at the far right (0 ppm) of the spectrum corresponds to the 12 chemically equivalent protons in tetramethylsilane (TMS) (Figure 29.9c.), a standard reference compound that was added to the sample. Very few organic molecules contain protons with chemical shifts that are negative relative to TMS.



tetramethylsilane (TMS)

Figure 29.9c. Structure of TMS (credit: *Organic Chem: Biological Emphasis vol. 1 (page 244)*, CC BY-NC-SA 4.0).

The two proton groups in our methyl ethanoate sample are recorded as resonating at frequencies 2.05 and 3.67 ppm higher than TMS. Expressed this way, the resonance frequency for a given proton in a molecule is called its chemical shift. A frequently used symbolic designation for chemical shift in ppm is the lower-case Greek letter delta (δ). Most protons in organic compounds have chemical shift values between 0 and 12 ppm from TMS, although values below zero and above 12 are occasionally observed. By convention, the left-hand side of an NMR spectrum (higher chemical shift) is called downfield, and the right-hand direction is called upfield.

Why do nonequivalent protons have different chemical shifts? The chemical shift of a given proton is determined primarily by its immediate electronic environment. Consider fluoromethane fluoride, CH_3F, in which the protons have a chemical shift of 4.26 ppm, significantly higher than that of methane at 0.23 ppm. Because fluorine is more electronegative than carbon, it pulls valence electrons away from the carbon, effectively decreasing the electron density around each of the protons. For the protons, lower electron density means less shielding and lower chemical shift. As the electronegativity of the substituent increases, so does

the extent of deshielding, and so does the chemical shift. This is evident in Figure 29.9d. with the chemical shifts of methane and three halomethane compounds. As the number of electronegative substituents increases in a compound, the more deshielding that happens and the greater the chemical shift. The deshielding effect of an electronegative substituent diminishes sharply with increasing distance.

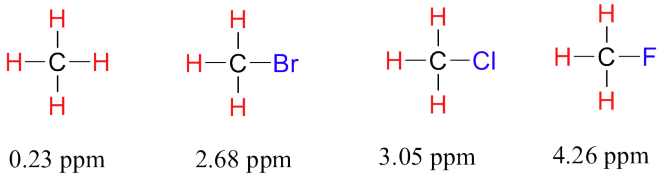


Figure 29.9d. Chemical shift of various halomethanes. Increase in chemical shift seen with increase in electronegativity (F more electronegative Cl which is more than Br which is more than H) (credit: *Organic Chem: Biological Emphasis vol. 1 (page 248)*, CC BY-NC-SA 4.0).

Armed with this information, the two peaks in the ^{1}H-NMR spectrum (Figure 29.9b.) of methyl ethanoate can be identified (Figure 29.9e.). The signal at 3.65 ppm corresponds to the THREE methyl protons (H_b), which are deshielded by the adjacent oxygen atom. The signal at 2.05 ppm corresponds to the THREE ethanoate protons (H_a), which is deshielded – but to a lesser extent – by the adjacent carbonyl group.

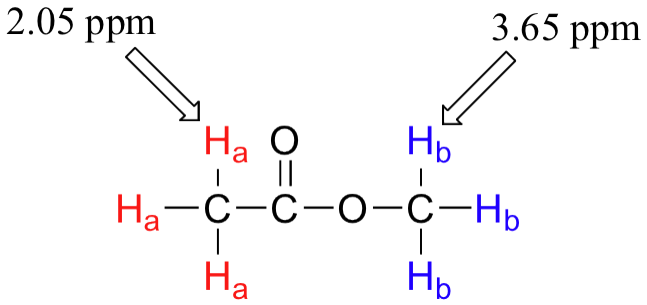


Figure 29.9e. Identification of protons in methyl ethanoate (Credit: *Organic Chemistry with a Biological Emphasis Volume I (page 249)*, CC BY-NC-SA 4.0, edited by *Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

The ability to recognize chemical equivalency and nonequivalency among atoms in a molecule will be central to understanding NMR. In each of the molecules in Figure 29.9f., all protons are chemically equivalent, and therefore will have the same resonance frequency in an NMR experiment.

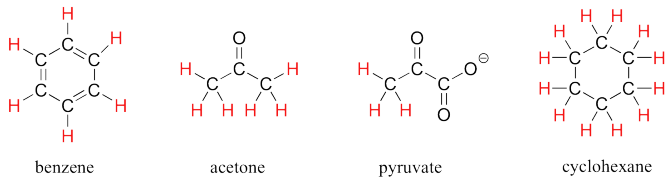


Figure 29.9f. Equivalent protons in benzene, acetone (propanone), pyruvate, and cyclohexane (Credit: *Organic Chemistry with a Biological Emphasis Volume I (page 239)*, CC BY-NC-SA 4.0, edited by *Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

Each the molecules in Figure 29.9g. contains two sets of protons, just like the previous example of methyl ethanoate, and again in each case the resonance frequency of the H_a protons will be different from that of the H_b protons. Notice how the symmetry of para-xylene results in there being only two different sets of protons.

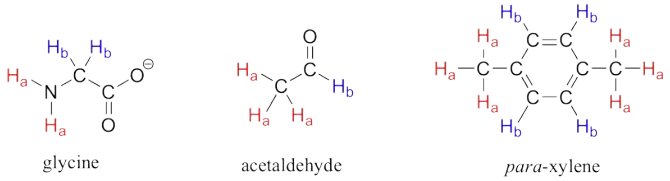


Figure 29.9g. Equivalent protons in glycine, ethanal (acetaldehyde), and para-dimethylbenzene (xylene) (credit: *Organic Chem: Biological Emphasis vol. 1 (page 239)*, CC BY-NC-SA 4.0).

Most organic molecules (Figures 29.9h. and 29.9i.) have several sets of protons in different chemical environments, and each set, in theory, will have a different resonance frequency in ^{1}H-NMR spectroscopy.

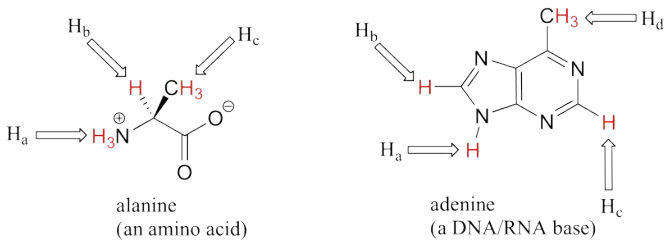


Figure 29.9h. Equivalent protons in alanine and adenine (credit: *Organic Chem: Biological Emphasis vol. 1 (page 241)*, CC BY-NC-SA 4.0).

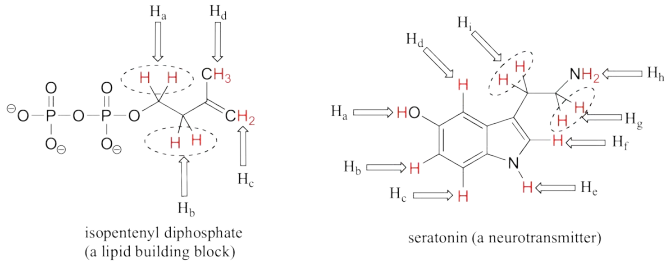
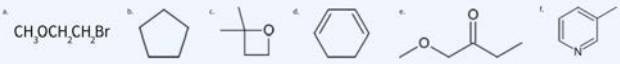


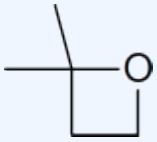
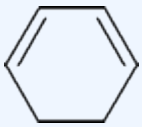
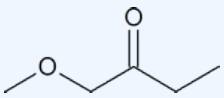
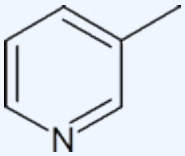
Figure 29.9i. Equivalent protons in isopentyl diphosphate and seratonin (credit: *Organic Chem: Biological Emphasis vol. 1 (page 241)*, CC BY-NC-SA 4.0).

Example 29.9a

Identify the number different hydrogen signals in each compound and list the number of hydrogen atoms making each signal.



Solutions

Structure	Number of Signals	Ratio of Signals		Reasoning
$CH_3OCH_2CH_2Br$	3	A : B : C	3 : 2 : 2	Each CH_2 group is located next to different ele atoms so their shifts will be different.
	1			All H's are equivalent. Each carbon (point) ha Total of 10 H's in the compound.
	3	A : B : C	2 : 2 : 6 (or 1 : 1 : 3)	The two CH_3 groups in the left are equivalent carbon on the lower left has 2 H's. These are d the 2 H's on the lower right carbon (which is n electronegative O atom).
	3	A : B : C	2 : 4 : 2 (or 1 : 2 : 1)	At the top of the ring, there is one H attached carbon. These two H's are connected in the sa double bonds so create one signal. Each carbo end of the double bonds has one H. These cre signal. The two carbons at the bottom of the ri H's each. These four H's create one signal.
	4	A : B : C : D	3 : 2 : 2 : 3	Starting on the right, the CH_3 group is one sig next to it is another group (next to carbonyl gr the O and the carbonyl group is another CH_2 different as it's next to two electronegative grou CH_3 group attached to the O atom is the final
	5	A : B : C : D : E	3 : 1 : 1 : 1 : 1	The CH_3 group is one signal. Each carbon in one H except where the methyl group is attach attached to the ring is a different distance from well as the double bond presence, so each creat signal.

Source: Example 29.9a is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Exercise 29.9a

How many non-equivalent hydrogens are in the following molecules? (How many different signals will be seen in a ^{1}H NMR spectrum?)

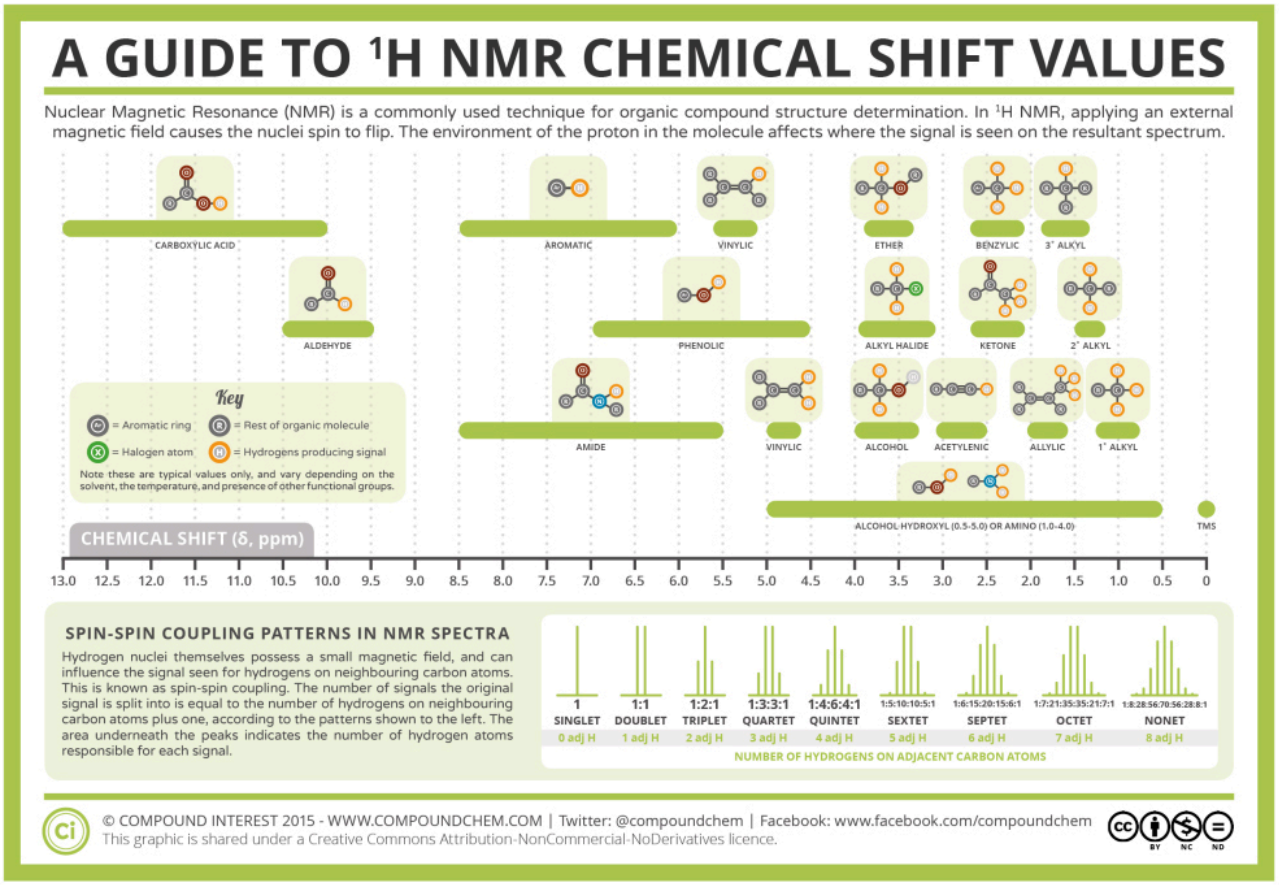
a. $CH_3CH_2CH_2Br$
b. $CH_3OCH_2C(CH_3)_3$
c. Ethyl Benzene
d. 2-methyl-1-hexene

Check Your Answers: [1]

Source: Exercise 29.9a is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Chemical Shifts in ^{1}NMR Spectrum

It is important to understand trend of chemical shift in terms of NMR interpretation. The proton NMR chemical shift is affected by nearness to electronegative atoms (O, N, halogen) and unsaturated groups (C=C, C=O, aromatic). ^{1}H chemical shifts play a role in identifying many functional groups. Infographic 29.9a. and Figure 29.9j. indicate chemical shifts of important functional groups. Tetramethylsilane (TMS) is generally used for standard to determine chemical shift of compounds: δ_{TMS}=0ppm. In other words, frequencies for chemicals are measured for a ^{1}H or ^{13}C nucleus of a sample from the ^{1}H or ^{13}C resonance of TMS.



Infographic 29.9a. 1H NMR chemical shifts. Read more about "Analytical Chemistry – A Guide to Proton Nuclear Magnetic Resonance (NMR) (https://www.compoundchem.com/2015/02/24/proton-nmr/)" by Andy Brunning / Compound Interest, CC BY-NC-ND, or access a text-based summary of infographic 29.9a [New tab].

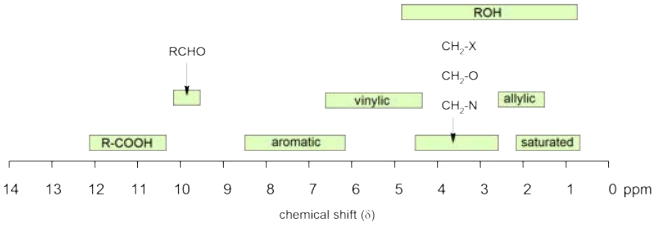
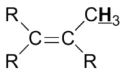
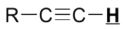
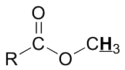
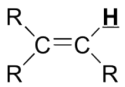
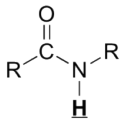
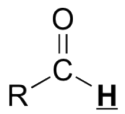
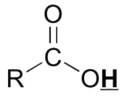


Figure 29.9j. Chemical shifts for proton NMR of functional groups (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

Table 29.9a. lists the chemical shift values are in parts per million (ppm) relative to TMS. The listed values may vary slightly from Figure 29.9j. and Infographic 29.9a. Note, Hydrogen (H) is underlined in each hydrogen type.

Table 29.9a. ^{1}H NMR Chemical shifts of functional groups

Hydrogen type[2]	Chemical shift (ppm)
$RC\underline{\mathbf{H}}_3$	0.9 – 1.0
$RC\underline{\mathbf{H}}_2R$	1.2 – 1.7
$R_3C\underline{\mathbf{H}}$	1.5 – 2.0
	1.5 – 1.8
$RN\underline{\mathbf{H}}_2$	1 – 3
$ArC\underline{\mathbf{H}}_3$	2.2 – 2.4
	2.3 – 3.0
$ROC\underline{\mathbf{H}}_3$	3.7 – 3.9
	3.7 – 3.9
$RO\underline{\mathbf{H}}$	1 – 5
	3.7 – 6.5
	5 – 9
$Ar\underline{\mathbf{H}}$	6.0 – 8.7
	9.5 – 10.0

Hydrogen type[2]	Chemical shift (ppm)
	10 – 13

Source: "12.5: Functional Groups and Chemical Shifts in ^{1}H NMR Spectroscopy" In *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Watch How to Identify Molecules – Proton NMR: Crash Course Organic Chemistry #26 on YouTube (11 mins) (https://www.youtube.com/watch?v=Liit4blsrqM)

Video source: Crash Course. (2021, April 21). *How to identify molecules – Proton NMR: Crash Course Organic Chemistry #26* [Video]. YouTube.

Example 29.9b

How do different functional groups (of comparable compounds) show in NMR spectra? Use Figure 29.9k to help.

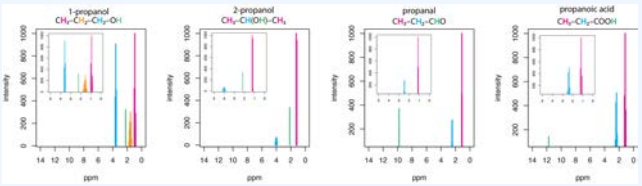


Figure 29.9k. ^{1}H NMR spectra for 1-propanol, 2-propanol, propanal, and propanoic acid. The full spectrum are shown using the scale at the bottom of each figure. The insets show close-ups of the NMR spectra from 0 – 5 ppm. The original data used to construct these spectra are found in the Spectral Database for Organic Compounds, SDBS [New tab] (https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi). (Credit: *Instrumental Analysis*, CC BY-NC-SA 4.0).

Solution

The spectra in Figure 29.9k. are for a set of four simple organic molecules, each of which has a chain of three carbons and an oxygen: 1-propanol, $CH_3CH_2CH_2OH$, 2-propanol, $CH_3CH(OH)CH_3$, propanal, CH_3CH_2CHO, and propanoic acid, CH_3CH_2COOH. The first two of these molecules are alcohols, the third is an aldehyde, and the last is a carboxylic acid. The main spectrum runs from 0–14 ppm, with insets showing the spectra over a narrower range of 0–5 ppm.

Each of these molecules has a terminal $-CH_3$ group that is the most upfield peak in its spectrum, appearing between 0.94 – 1.20 ppm. Each of these molecules has a hydrogen that either is bonded to

an oxygen or a hydrogen bonded to the same carbon as the oxygen. The hydrogens in the –OH groups of the two alcohols have similar shifts of 2.16 ppm and 2.26 ppm, but the aldehyde hydrogen in the –CHO group and the acid hydrogen in –COOH are shifted further downfield appearing at 9.793 ppm and 11.73 ppm, respectively. The hydrogens in the two $–CH_2–$ groups of 1-propanol have very different shifts, with the one adjacent to the –OH group appearing more downfield at 3.582 ppm than the one next to the $–CH_3$ group at 1.57 ppm. Not surprisingly, the –CH– hydrogen in 2-proponal, which is adjacent to the –OH group appears at 4.008 ppm.

Example source: *Instrumental Analysis (LibreTexts)*, CC BY-NC-SA 4.0

Exercise 29.9b

What would the ^{1}H NMR spectrum of this compound look like?

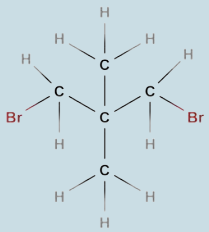


Figure 29.9l. Structure of 1,3-dibromo-2,2-dimethylpropane (credit: Image by Samantha Sullivan Sauer using MolView, CC BY-NC 4.0)

Check Your Answers: [3]

Source: Exercise created by Samantha Sullivan Sauer, CC BY-NC 4.0

Signal Integration

The concept of peak integration is that the area of a given peak in a ^{1}H NMR spectrum is proportional to the

number of (equivalent) protons giving rise to the peak. Thus, a peak which is caused by a single, unique proton has an area which measures one third of the area of a peak resulting from a methyl (CH_3) group in the same spectrum.

In practice, we do not have to measure these areas ourselves: it is all done electronically by the spectrometer, and an integration curve is superimposed on the rest of the spectrum. The integration curve appears as a series of steps, with the height of each step being proportional to the area of the corresponding absorption peak, and consequently, to the number of protons (hydrogens) responsible for the absorption. The two signals in the methyl acetate spectrum, for example, integrate to approximately the same area, because they both correspond to a set of three equivalent protons.

Example 29.9c

Figure 29.9m. is the spectrum of para-xylene (IUPAC name 1,4-dimethylbenzene). Explain the equivalent protons and the integration results.

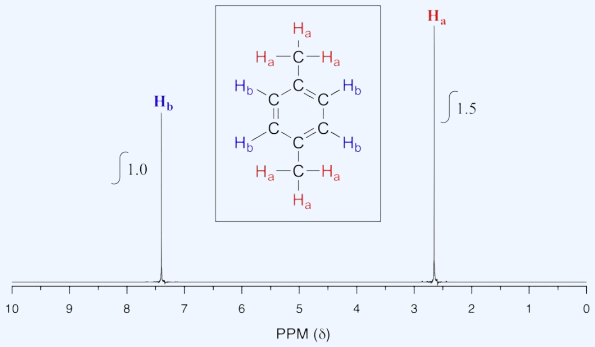


Figure 29.9m. NMR spectrum of para-xylene (IUPAC name 1,4-dimethylbenzene) (credit: *Organic Chem: Biological Emphasis vol. 1 (page 246)*, CC BY-NC-SA 4.0).

Solution

This molecule has two sets of protons: the six methyl (H_a) protons and the four aromatic (H_b) protons. When the instrument integrates the areas under the two signals, we find that the area under the peak at 2.6 ppm is 1.5 times greater than the area under the peak at 7.4 ppm. This along with the actual chemical shift values tells us which set of protons corresponds to which NMR signal. The peak at 2.6 ppm represents the 6 methyl protons because they are attached to the aromatic ring (Ar-CH_3) and the peak at 7.4 ppm represents the four aromatic protons because they are directly attached to the aromatic ring (Ar-H).

Source: Example is adapted from *Organic Chem: Biological Emphasis Vol I (page 246)*, CC BY-NC-SA 4.0.

The integration function can also be used to determine the relative amounts of two or more compounds in a mixed sample. If a sample is a 50:50 (mole/mole) mixture of benzene and acetone, for example, the acetone signal should integrate to the same value as the benzene sample, because both signals represent six equivalent protons. If a sample is a 50:50 mixture of acetone and cyclopentane, on the other hand, the ratio of the acetone peak area to the cyclopentane peak area will be 3:5 (or 6:10), because the cyclopentane signal represents ten protons.

Exercise 29.9c

A ^{1}H-NMR spectrum of a mixed 50:50 (mole: mole) sample of acetone (propanone) ($CH_3(CO)CH_3$) and dichloromethane (CH_2Cl_2). What is the ratio of the acetone peak area to the dichloromethane peak area?

Check Your Answer: [4]

Source: Exercise is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Exercise 29.9d

The $_{1}$H-NMR spectrum of a mixed sample of 50% para-xylene and 50% acetone in $CDCl_3$ solvent (structures are shown in Figure 29.9f. and 29.9g.). How many peaks do you expect to see? What is the expected ratio of integration values for these peaks?

Check Your Answer: [5]

Source: Exercise is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Spin-Spin Coupling

The ^{1}H-NMR spectra seen in Figures 29.9b. and 29.9m. (of methyl acetate and *para*-xylene) are somewhat unusual in the sense that in both of these molecules, each set of protons generates a single NMR signal. In fact, the ^{1}H-NMR spectra of most organic molecules contain proton signals that are 'split' into two or more sub-peaks. Rather than being a complication, however, this splitting behavior actually provides a lot of information about the sample molecule.

Consider the spectrum for 1,1,2-trichloroethane in Figure 29.9n. In this and in many spectra to follow, we show enlargements of individual signals so that the signal splitting patterns are recognizable.

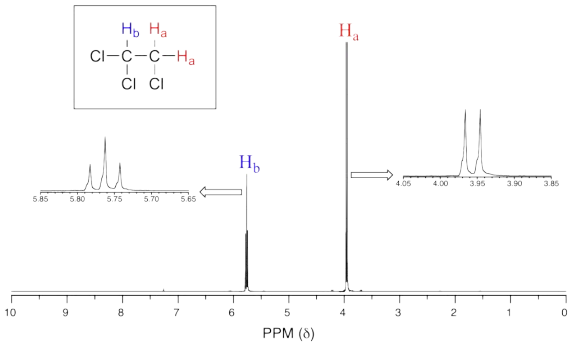


Figure 29.9n. 1H NMR spectrum of 1,1,2-trichloroethane showing spin-spin coupling (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

From what we have learned about ^{1}H NMR spectra so far, we might predict that the spectrum of 1,1,2-trichloroethane, $CHCl_2CH_2Cl$, would consist of two peaks—one, at about 2.5-4.0 δ, expected for CH_2-halogen compounds and one shifted downfield because of the presence of an additional electronegative chlorine atom on the second carbon. The signal at 3.96 ppm, corresponding to the two H_a protons, is split into two subpeaks of equal height (and area) – this is referred to as a doublet. The H_b signal at 5.76 ppm, on the other hand, is split into three sub-peaks, with the middle peak double the area of each of the two outside peaks. This is called a triplet. The split peaks (multiplets) arise because the magnetic field experienced by the protons of one group is influenced by the spin arrangements of the protons in an adjacent group. Spin-spin coupling is often referred to as spin-spin splitting and is based on the $n + 1$ rule and the associated coupling patterns.

The source of signal splitting is a phenomenon called spin-spin coupling, a term that describes the magnetic interactions between neighbouring, non-equivalent NMR-active nuclei. In 1,1,2-trichloromethane, the H_a and H_b protons are spin-coupled to each other. Looking first at the H_a signal: in addition to being shielded by nearby valence electrons, each of the H_a protons is also influenced by the small magnetic field generated by H_b next door (remember, each spinning proton is like a tiny magnet). The interaction 'felt' by H_a is a slightly weaker if H_b is aligned against B_0, or slightly stronger if H_b is aligned with B_0. In other words, in half of the molecules, H_a is shielded by H_b (thus the NMR signal is shifted slightly upfield) and in the

other half H_a is deshielded by H_b(and the NMR signal shifted slightly downfield). What would otherwise be a single H_a peak has been split into two sub-peaks (a doublet), one upfield and one downfield of the original signal. These ideas can be illustrated by a splitting diagram, as shown in Figure 29.9o.

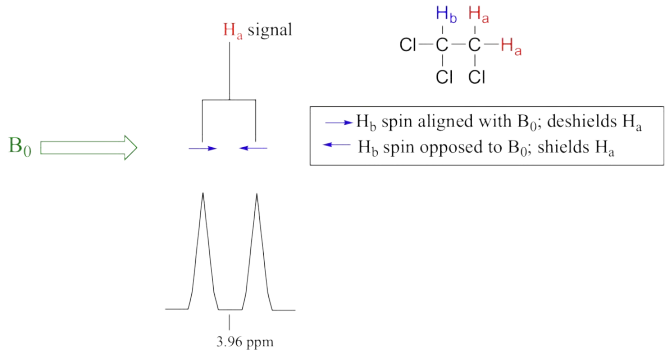


Figure 29.9o. The spin-spin coupling between Ha and Hb in 1,1,2-trichloroethane (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

A similar explanation now applies to the H_b signal. The magnetic environment experienced by H_b is influenced by the fields of both neighbouring H_a protons, called H_{a1} and H_{a2}. There are four possibilities here, each of which is equally probable. First, the magnetic fields of both H_{a1} and H_{a2} could be aligned with B_0, which would deshield H_b, shifting its NMR signal slightly downfield. Second, both the H_{a1} and H_{a2} magnetic fields could be aligned opposed to B_0, which would shield H_b, shifting its resonance signal slightly upfield. Third and fourth, H_{a1} could be with B_0 and H_{a2} opposed, or H_{a1} opposed to B_0 and H_{a2} with B_0. In each of the last two cases, the shielding effect of one H_a proton would cancel the deshielding effect of the other, and the chemical shift of H_b would be unchanged. In the end, the signal for H_b is a triplet (Figure 29.9p), with the middle peak twice as large as the two outer peaks because there are two ways that H_{a1} and H_{a2} can cancel each other out.

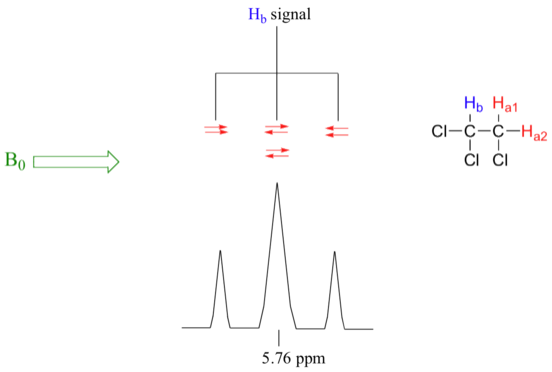


Figure 29.9p. The spin-spin coupling between Hb and Ha in 1,1,2-trichloroethane (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

Example 29.9d

Explain the spin-spin coupling in the NMR spectrum (Figure 29.9q.) for ethyl ethanoate.

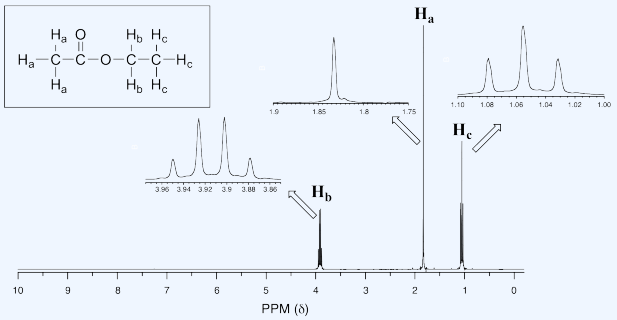


Figure 29.9q. NMR spectrum of ethyl ethanoate (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)..

Solution

We see an unsplit 'singlet' peak at 1.833 ppm that corresponds to the ethanoate (H_a) hydrogens – this is similar to the signal for the ethanoate hydrogens in methyl ethanoate (Figure 29.9b). This signal is unsplit because there are no adjacent hydrogens on the molecule. The signal at 1.055 ppm for the H_C hydrogens is split into a triplet by the two H_b hydrogens next door. The explanation here is the same as the explanation for the triplet peak seen in Figure 29.9m. The H_b hydrogens give rise to a quartet signal at 3.915 ppm – notice that the two middle peaks are taller than the two outside peaks. This splitting pattern results from the spin-coupling effect of the three H_C hydrogens next door and can be explained by an analysis similar to that used to explain the doublet and triplet patterns.

Source: Example is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

The spin-spin coupling pattern is usually referred to as the $n + 1$ rule: if a set of hydrogens has n neighbouring, non-equivalent hydrogens, it will be split into $n + 1$ subpeaks. Thus, the two H_b hydrogens in ethyl ethanoate split the H_c signal into a triplet, and the three H_c hydrogens split the H_b signal into a quartet. This is very useful information if trying to determine the structure of an unknown molecule: a triplet signal means that the corresponding hydrogen or set of hydrogens has two neighbours.

Keep in mind that:

1. Signal splitting only occurs between non-equivalent hydrogens – in other words, H_{a1} in 1,1,2-trichloroethane is not split by H_{a2}, and vice-versa.
2. Splitting occurs primarily between hydrogens that are separated by three bonds. This is why the H_a hydrogens in ethyl ethanoate form a singlet – the nearest hydrogen neighbours are five bonds away, too

far for coupling to occur.

3. Splitting is most noticeable with hydrogens bonded to carbon. Hydrogens that are bonded to heteroatoms (alcohol or amino hydrogens, for example) are coupled weakly – or not at all – to their neighbours.

Figures 29.9r. and 29.9s. are a few more examples of chemical shift and splitting pattern information for some relatively simple organic molecules.

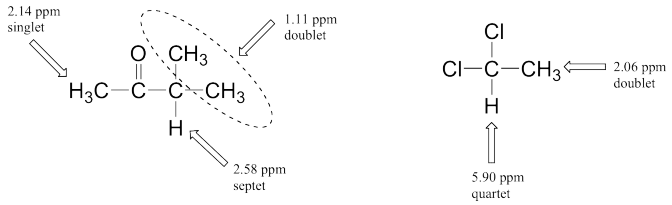


Figure 29.9r. The spin-spin coupling of two simple organic molecules (credit: *Organic Chem: Biological Emphasis vol. 1 (page 258)*, CC BY-NC-SA 4.0).

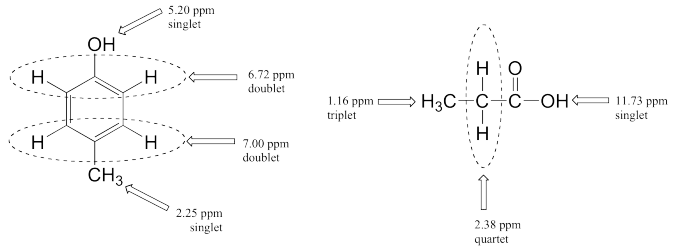


Figure 29.9s. The spin-spin coupling of two simple organic molecules (credit: *Organic Chem: Biological Emphasis vol. 1 (page 258)*, CC BY-NC-SA 4.0).

Exercise 29.9e

How many proton signals would be expected in the ^{1}H-NMR spectrum of triclosan (a common antimicrobial agent found in detergents)? For each of the proton signals, predict the splitting pattern.

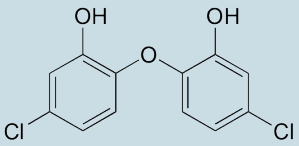


Figure 29.9t. Structure of triclosan (credit: *Organic Chem: Biological Emphasis vol. 1 (page 258)*, CC BY-NC-SA 4.0).

Check Your Answers:[6]

Source: Exercise is adapted from *Organic Chem: Biological Emphasis Vol I (page 258)*, CC BY-NC-SA 4.0.

Links to Enhanced Learning

The Chemical Detectives app on Chemical Detectives – Apps on Google Play [New tab] (https://play.google.com/store/apps/details?id=com.chemicaldetectives&pli=1) and Chemical Detectives on the App Store [New tab] (https://apps.apple.com/au/app/chemical-detectives/id741760895) allows users to browse various types of spectra (IR, MS, ^{1}H NMR, ^{13}C NMR, and elemental microanalysis) for simple organic compounds and also complete quizzes about various compounds based on their spectra. Customize of types of functional groups is possible (e.g. only hydrocarbons or only alcohols).

Other options:

- Predict 1H proton NMR spectra [New tab] (https://www.nmrdb.org/new_predictor/index.shtml?v=v2.138.0)
- Searchable database of spectra AIST:Spectral Database for Organic Compounds,SDBS [New tab] (https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi)

Attribution & References

Except where otherwise noted, this section has been adapted by Samantha Sullivan Sauer from:

- "19.4: Applications of Proton NMR" by David Harvey In *Instrumental Analysis (LibreTexts)* , CC BY-NC-SA 4.0
- "12.7: Spin-Spin Splitting in ^{1}H NMR Spectra" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II*, CC BY-NC-SA 4.0. Contributors from original source:
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  - Prof. Steven Farmer (Sonoma State University (http://www.sonoma.edu))
  - Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)
  - Chris P Schaller, Ph.D. (http://employees.csbsju.edu/cschaller/srobi.htm), (College of Saint Benedict / Saint John's University) (http://www.csbsju.edu/Chemistry.htm)
- "12.2: NMR Spectra – an introduction and overview", "12.4: ^{1}H NMR Spectroscopy and Proton Equivalence", "12.5: Functional Groups and Chemical Shifts in ^{1}H NMR Spectroscopy", "12.6: Integration of ^{1}H NMR Absorptions- Proton Counting" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II* by LibreTexts, licensed under CC BY-NC-SA 4.0. Contributors from original source:
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  - Prof. Steven Farmer (Sonoma State University (http://www.sonoma.edu))
  - Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

Notes

1. A. 3; B. 3; C. 5; D. 7

2. R stands for any alkyl chain; Ar stands for any aryl group (benzene based).
3. The spectrum will have two signals. One represents the two CH_3 groups totaling 6 Hs. This will be about 0.9-1.0 ppm. The other represents the two CH_2 groups next to the Br atoms totaling 4 Hs. This will be about 3.4-3.6

4. Acetone has 6 equivalent protons. Dichloromethane has 2 equivalent protons. This is a 6:2 or 3:1 ratio.

5. para-xylene has two peaks with a 6:4 ratio. Acetone has one peak representing 6 protons. The ratio between all peaks in this 50:50 mixture is 6:4:6 or 3:2:3.

6. There will be four signals in the spectrum. Each peak represents two hydrogens given the symmetry of the molecule. The H on the OH and the H on the ring between OH and Cl will be single peaks. The other two Hs will be doublets due to the neighbouring Hs.

29.10 ^{13}C NMR SPECTROSCOPY

Learning Objectives

By the end of this section, you will be able to:

- Identify the differences between ^{1}H NMR and ^{13}C NMR and their spectra.
- Identify those carbon atoms which are equivalent (due to symmetry) in a given chemical structure.
- Predict the approximate chemical shifts of each of the carbons in an organic compound, given its structure and a table of chemical shift correlations.

Building on the principles of NMR explained in Section 29.8 and the interpretation of ^{1}H NMR in Section 29.9, this section focuses on the interpretation of ^{13} C NMR.

Differences between ^{1}H and ^{13}C NMR Spectroscopy

The magnetic moment of a ^{13}C nucleus is much weaker than that of a proton, meaning that NMR signals from ^{13}C nuclei are inherently much weaker than proton signals. This, combined with the low natural abundance of ^{13}C, means that it is much more difficult to observe carbon signals: more sample is required, and often the data from hundreds of scans must be averaged in order to bring the signal-to-noise ratio down to acceptable levels.

Unlike ^{1}H-NMR signals, the area under a ^{13}C-NMR signal cannot be used to determine the number of carbons to which it corresponds. This is because the signals for some types of carbons are inherently weaker than for other types – peaks corresponding to carbonyl carbons, for example, are much smaller than those for methyl or methylene (CH_2) peaks.

Just like in ^{1}H-NMR, the standard used in ^{13}C-NMR experiments to define the 0 ppm point is tetramethylsilane (TMS), although of course in ^{13}C-NMR it is the signal from the four equivalent carbons in TMS that serves as the standard. Chemical shifts for ^{13}C nuclei in organic molecules are spread out over a much wider range than for protons – up to 200 ppm for ^{13}C compared to 12 ppm for protons. This is also

fortunate, because it means that the signal from each carbon in a compound can almost always be seen as a distinct peak, without the overlapping that often plagues ^{1}H-NMR spectra. The chemical shift of a ^{13}C nucleus is influenced by essentially the same factors that influence a proton's chemical shift: bonds to electronegative atoms and diamagnetic anisotropy effects tend to shift signals downfield (higher resonance frequency). In addition, sp^2 hybridization results in a large downfield shift. The ^{13}C-NMR signals for carbonyl carbons are generally the furthest downfield (170-220 ppm), due to both sp^2 hybridization and to the double bond to oxygen.

Because of the low natural abundance of ^{13}C nuclei, it is very unlikely to find two ^{13}C atoms near each other in the same molecule, and thus we do not see spin-spin coupling between neighbouring carbons in a ^{13}C-NMR spectrum. Figure 29.10a. is the ^{13}C-NMR spectrum of ethyl ethanoate, showing the expected four signals, one for each of the carbons.

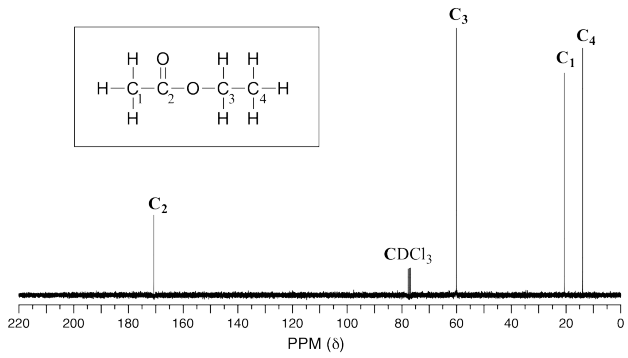


Figure 29.10a. 13C-NMR spectrum of ethyl ethanoate, showing the expected four signals, one for each of the carbons (credit: *Organic Chem: Biological Emphasis Vol I (page 265)*, CC BY-NC-SA 4.0).

Symmetry in Molecules

In the ^{13}C NMR spectrum of pentane (Figure 29.10b.), there are three different peaks, even though pentane just contains methyl carbons (CH_3) and methylene carbons (CH_2). As far as the NMR spectrometer is concerned, pentane contains three different kinds of carbon, in three different environments. That result comes from symmetry.

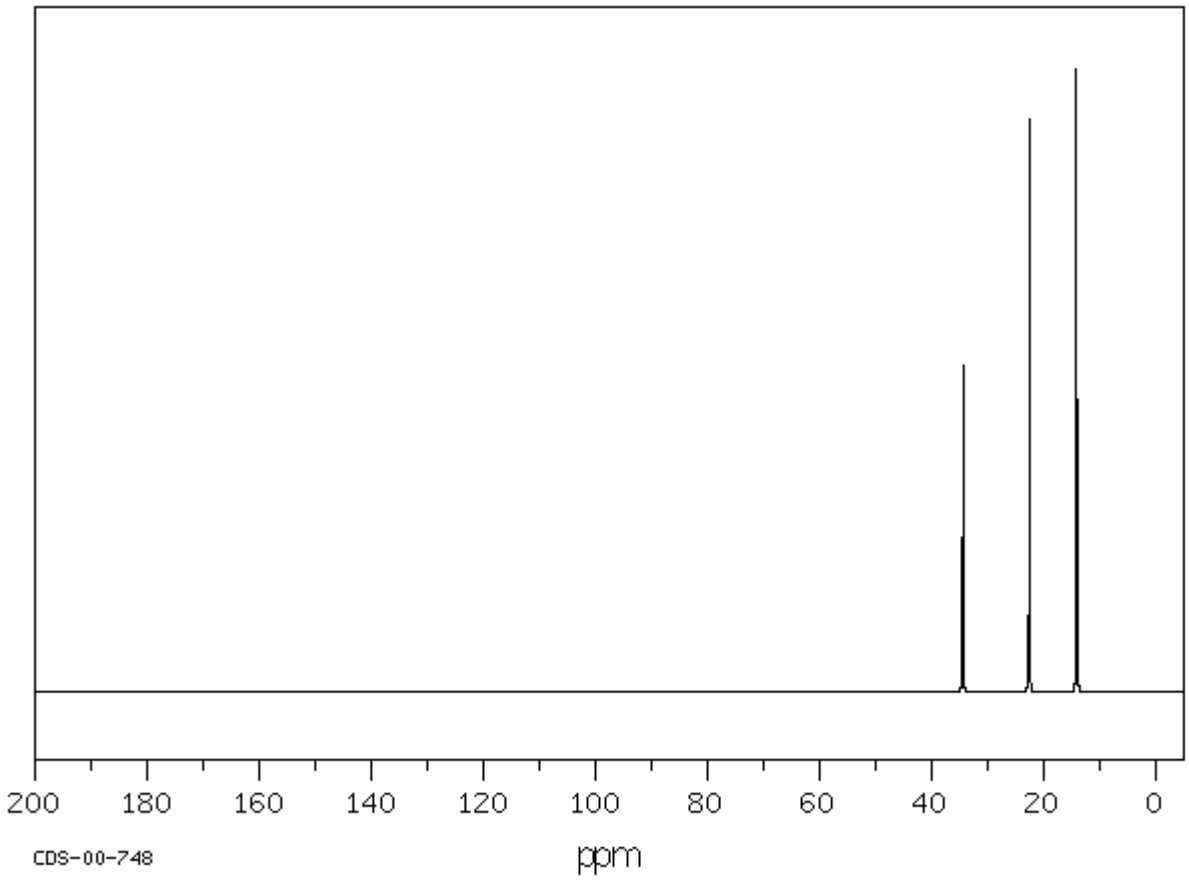


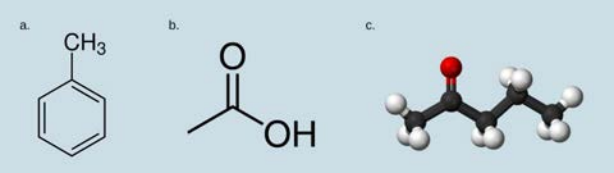
Figure 29.10b. ^{13}C NMR spectrum of pentane. (**Credit:** "Pentane– compound 2475" © National Institute of Advanced Industrial Science and Technology via SDBSWeb , accessed 11/21/2023.)

Symmetry is an important factor in spectroscopy. Carbon atoms that are symmetry-inequivalent can absorb at different shifts. Carbon atoms that are symmetry-equivalent must absorb at the same shift. In Figure 29.10c., the pentane molecule is shown in two different orientations where it is visible that the end carbons are symmetry-equivalent and the second and fourth carbons along the chain are also symmetry-equivalent. However, the middle carbon is not; it never switches places with the other carbons when rotating the model. There are three different sets of inequivalent carbons; these three groups are not the same as each other according to symmetry.

Figure 29.10c. Two different views of the same molecule of pentane showing the molecule's symmetry. (credit: Image by Samanatha Sullivan Sauer, created with MolView, CC BY-NC 4.0)

Exercise 29.10a

How many sets of non-equivalent carbons in each molecule?



(credit a: Image by Wesalius, PD; b: Image by Jynto & Ben Mills, PD; c: Image by Interiot~commonswiki, edited by Calvero, PD).

Check Your Answers: [1]

Source: Exercise created by Samantha Sullivan Sauer, CC BY-NC 4.0

^{13}C NMR Chemical Shifts

The carbon NMR is used for determining functional groups using characteristic shift values. ^{13}C chemical shift is affect by electronegative effect and steric effect. If an H atom in an alkane (Figure 29.10d.) is replace by substituent X, electronegative atoms (O, N, halogen), α-carbon and β-carbon shift to downfield (left; increase in ppm) while γ-carbon shifts to upfield.

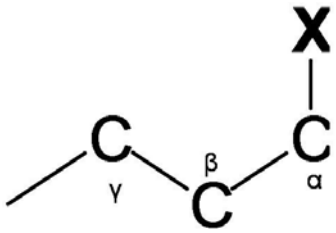
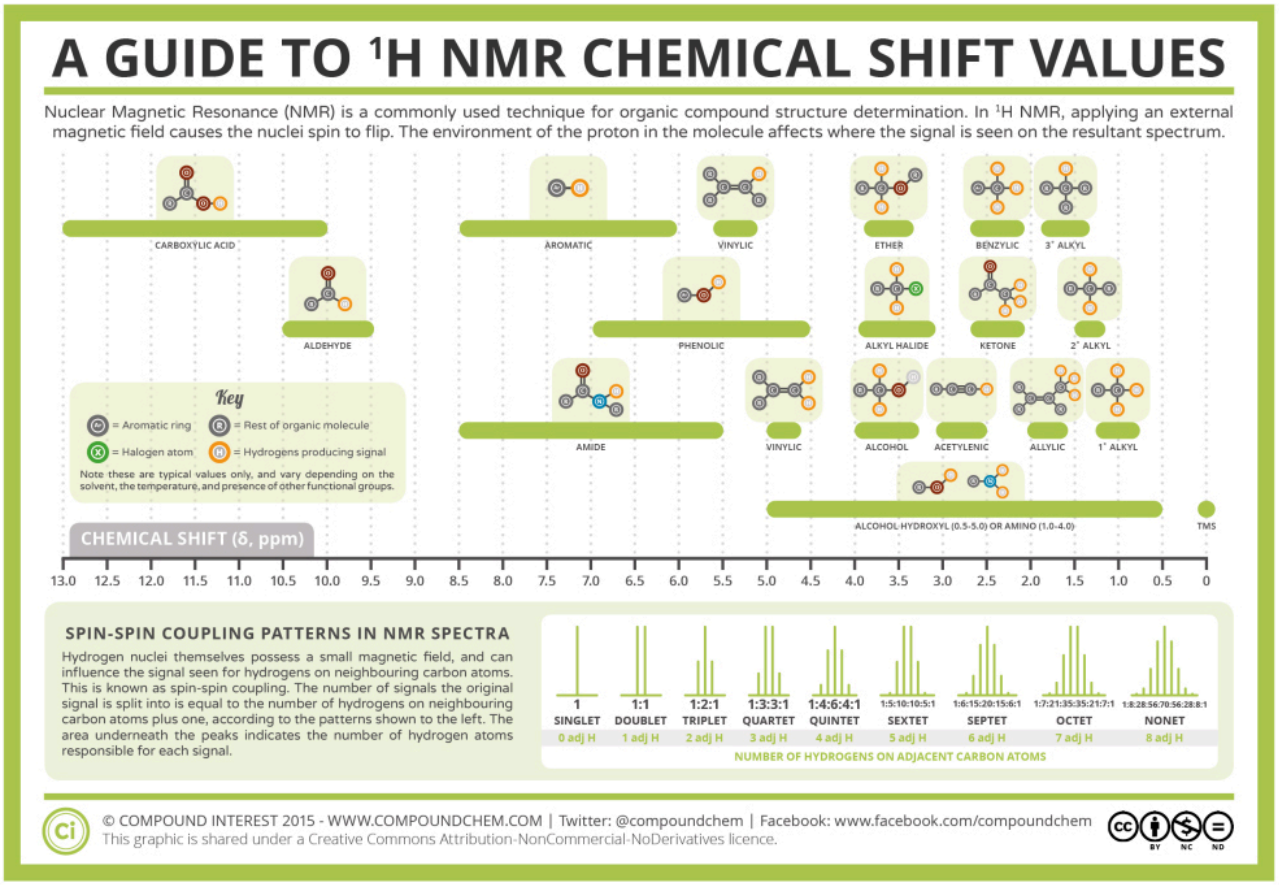


Figure 29.10d. X substituent on alpha carbon of alkane. Beta and gamma carbons are labelled (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

The steric effect is observed in acyclic and cyclic systems, which leads to downshifted chemical shifts. Infographic 29.10a and Figure 29.10e. show typical ^{13}C chemical shift regions of the major chemical classes.



Infographic 29.10a. Typical 13C chemical shift regions of the major chemical classes. Read more about "A Guide to 13-C Nuclear Magnetic Resonance (NMR) (https://www.compoundchem.com/2015/04/07/carbon-13-nmr/)" by Andy Brunning / Compound Interest, CC BY-NC-ND, or access a text-based summary of infographic 29.10a [New tab].

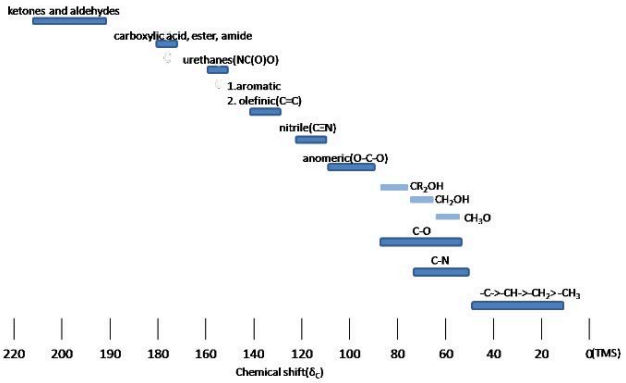


Figure 29.10e. Typical ^{13}C chemical shift regions of the major chemical classes (credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0).

Using the information in Infographic 29.10a and Figure 29.10e., peaks in the spectrum can be assigned.

Example 29.10a

Review the spectrum and chemical structure in Figure 29.10f. Justify the location of each peak to each carbon in the molecule.

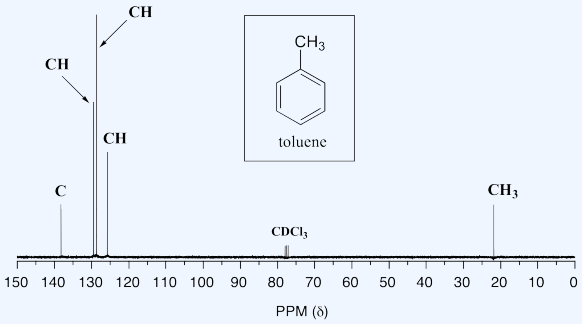


Figure 29.10f. ^{13}C NMR of methylbenzene (toluene) (credit: *Organic Chem: Biological Emphasis Vol I (page 266)*, CC BY-NC-SA 4.0).

Solution

The C peak at about 140 ppm corresponds to the carbon of the ring where the methyl group is attached. The three CH peaks between 125-130 ppm correspond to the carbons in the ring. Due to symmetry in the molecule, two of these peaks represents two carbons each, the third peak represents the carbon at the bottom of the ring. The CH_3 peak at about 23 ppm is the methyl group.

Source: Example is adapted from *Organic Chem: Biological Emphasis Vol I (page 266)*, CC BY-NC-SA 4.0.

Example 29.10b

Review the spectrum and chemical structure in Figure 29.10g. Justify the location of each peak to each carbon in the molecule.

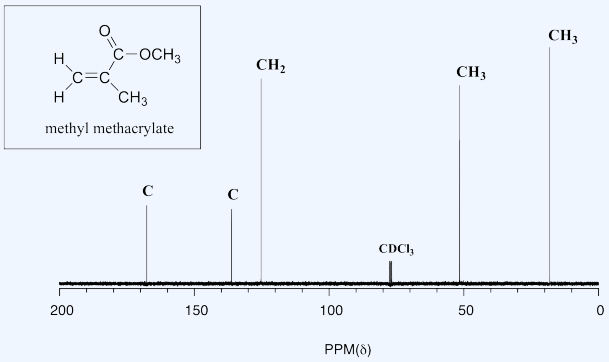


Figure 29.10g. ^{13}C NMR of methyl methacrylate (methyl 2-methylpro-2-penate) (credit: *Organic Chem: Biological Emphasis Vol I (page 266)*, CC BY-NC-SA 4.0).

Solution

The C peak about 170 ppm is from the ester carbon. The C peak about 128 ppm is from right hand carbon in the carbon-carbon double bond. The CH_2 peak about 125 ppm is from the left-hand carbon in the carbon-carbon double bond. The CH_3 peak about 50 ppm is from the methyl group of the ester (attached to the oxygen atom). Finally, the CH_3 peak about 20 ppm is from the methyl group attached to the carbon-carbon double bond.

Source: Example is adapted from *Organic Chem: Biological Emphasis Vol I (page 266)*, CC BY-NC-SA 4.0.

Exercise 29.10b

Explain the C-13 NMR spectrum of ethanol.

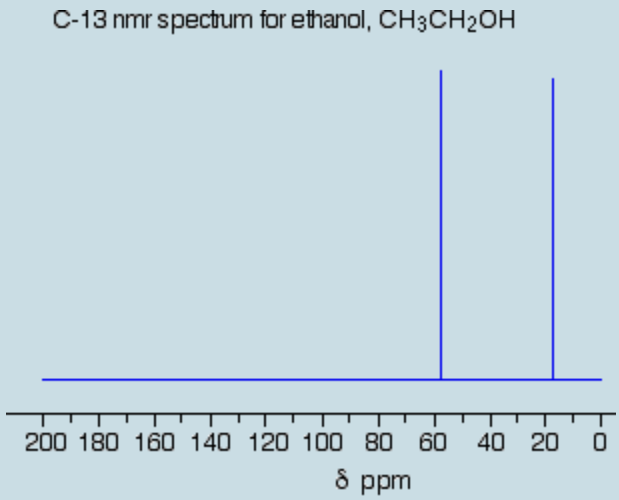


Figure 29.10h. ^{13}C NMR spectrum of ethanol. (Credit: "Ethyl Alcohol – compound 1300" © National Institute of Advanced Industrial Science and Technology via SDBSWeb, accessed 11/21/2023).

Check Your Answer: [2]

Source: Exercise is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Exercise 29.10c

Figures 29.10i. and 29.10j. show the structure and C-13 NMR spectrum for 1-methylethyl propanoate (also known as isopropyl propanoate or isopropyl propionate) respectively.

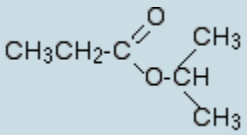


Figure 29.10i. Structure of 1-methylethyl propanoate

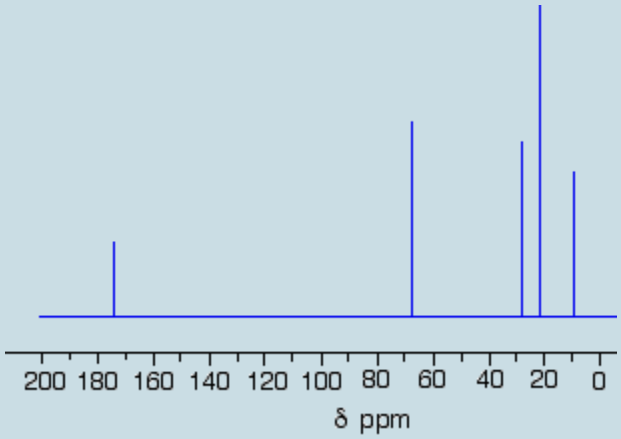


Figure 29.10j. ^{13}C NMR spectrum for 1-methylethyl propanoate. (Credit: "Isopropyl propionate – compound 2923" © National Institute of Advanced Industrial Science and Technology via SDBSWeb, accessed 11/21/2023)

There are 5 lines in the spectrum. That means that there must be 5 different environments for the carbon atoms in the compound. Is that reasonable from the structure?

Check Your Answers:[3]

Source: Exercise is adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

One of the greatest advantages of ^{13}C-NMR compared to ^{1}H-NMR is the breadth of the spectrum. In the proton NMR spectrum of 1-heptanol (Figure 29.10k.), only the signals for the alcohol proton (H_a) and the two protons on the adjacent carbon (H_b) are easily analyzed. The other proton signals overlap, making analysis difficult.

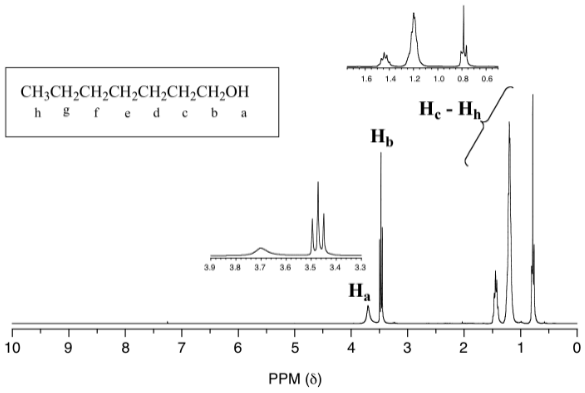


Figure 29.10k. Proton NMR spectrum of 1-heptanol (credit: *Organic Chem: Biological Emphasis Vol I (page 257)*, CC BY-NC-SA 4.0).

In the ^{13}C spectrum of the same molecule (Figure 29.10l.), however, each carbon signal can be easily distinguished, and we know from this data that our sample has seven non-equivalent carbons. As well, the chemical shifts of the carbons get progressively smaller as they get farther away from the oxygen atom. This property of ^{13}C-NMR makes it very helpful in the elucidation of larger, more complex structures.

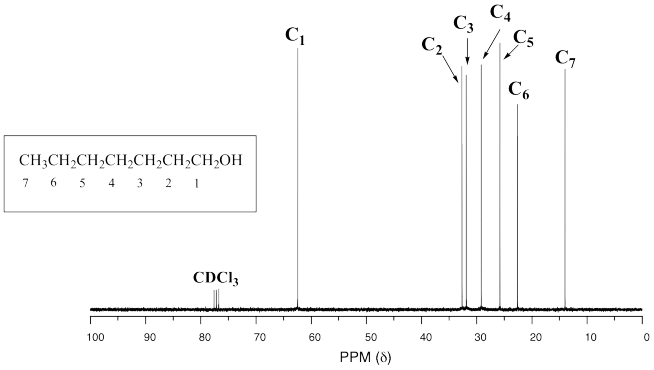


Figure 29.10l. Carbon NMR spectrum of 1-heptanol (credit: *Organic Chem: Biological Emphasis Vol I (page 266)*, CC BY-NC-SA 4.0).

Links to Enhanced Learning

The Chemical Detectives app on Chemical Detectives – Apps on Google Play and Chemical Detectives on the App Store (apple.com) allows users to browse various types of spectra (IR, MS, ^{1}H NMR, ^{13}C NMR, and elemental microanalysis) for simple organic compounds and also

complete quizzes about various compounds based on their spectra. Customize of types of functional groups is possible (e.g. only hydrocarbons or only alcohols).

Attribution & References

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  - William Reusch, Professor Emeritus (Michigan State U. (http://www.msu.edu/)), Virtual Textbook of Organic Chemistry (https://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/intro1.htm)
  - Organic Chemistry With a Biological Emphasis by Tim Soderberg (University of Minnesota, Morris)

Note: The NMR spectra on this page have been produced from graphs taken from the Spectral Data Base System for Organic Compounds (SDBS) at the National Institute of Materials and Chemical Research in Japan.

Notes

1. a) 5, b) 5, c) 2

2. There are two peaks because there are two different environments for the carbons. The carbon in the CH_3 group is attached to 3 hydrogens and a carbon. This peak is showing up around 20 ppm. The carbon in the CH_2 group is attached to 2 hydrogens, a carbon and an oxygen. This peak is showing up around 60 ppm. The two lines are in different places in the NMR spectrum because they need different external magnetic fields to bring them in to resonance at a particular radio frequency.

3. There are 6 carbon atoms but only 5 lines. Why? In this case, two of the carbons are in exactly the same environment. They are attached to exactly the same things. Look at the two CH_3 groups on the right-hand side of the molecule. Why the carbon in the CH3 on the left is not also in the same environment? Just like the ones on the right, the carbon is attached to 3 hydrogens and another carbon. But the similarity is not exact - follow the similarity along the rest of the molecule as well to be sure. The carbon in the left-hand CH_3 group is attached to a carbon atom which in turn is attached to a carbon with two oxygens on it - and so on down the molecule. That's not exactly the same environment as the carbons in the right-hand CH3 groups. They are attached to a carbon which is attached to a single oxygen - and so on down the molecule.

29.11 VISIBLE AND ULTRA-VIOLET SPECTROSCOPY (UV-VIS)

Learning Objectives

By the end of this section, you will be able to:

- Identify and describe conjugated double bonds.
- Differentiate between absorbance and transmittance.
- Describe the information available in UV-Vis spectrum.
- Identify some uses of UV-Vis spectroscopy.

Visible and ultraviolet (UV-Vis spectroscopy) uses electromagnetic radiation to detect the presence of conjugated double bonds and can be used to quantify concentrations of a compound in a sample.

Conjugated Double Bonds

Conjugated dienes are characterized by alternating carbon-carbon double bonds separated by carbon-carbon single bonds. Cumulated dienes are characterized by adjacent carbon-carbon double bonds (Figure 29.11a.). While conjugated dienes are energetically more stable than isolated double bonds, cumulated double bonds are unstable. Conjugated dienes are more stable than non-conjugated dienes (both isolated and cumulated) due to factors such as delocalization of charge through resonance and hybridization energy.

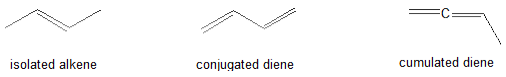

Figure 29.11a. Isolated, conjugated and cumulated carbon-carbon double bonds. (Credit: *Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)

The Visible-Ultraviolet Spectrum

The visible spectrum constitutes but a small part of the total electromagnetic radiation spectrum (see Chapter 29.5 Spectroscopy Basics). Most of the radiation that surrounds us cannot be seen but can be detected by dedicated sensing instruments. The energy associated with a given segment of the spectrum is proportional to its frequency and inversely proportional to its wavelength (high wavelength = low frequency). The visible portion of the spectrum is shown in Figure 29.11b.

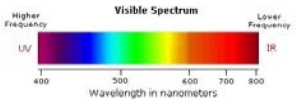

Figure 29.11b. Visible portion of electromagnetic spectrum. (Credit: *Virtual Textbook of Organic Chemistry*, CC BY-NC-SA 4.0)

When white light passes through or is reflected by a coloured substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary colour to the wavelength(s) absorbed. This relationship is demonstrated by the colour wheel shown in Figure 29.11c. Here, complementary colours are diametrically opposite each other. Thus, absorption of 420-430 nm light renders a substance yellow, and absorption of 500-520 nm light makes it red. Green is unique in that it can be created by absorption close to 400 nm as well as absorption near 800 nm.

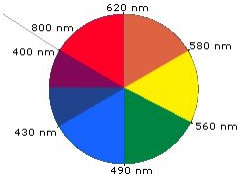

Figure 29.11c. Colour wheel showing complementary colours opposite each other. (Credit: *Virtual Textbook of Organic Chemistry*, CC BY-NC-SA 4.0)

Early humans valued coloured pigments and used them for decorative purposes. Many of these were inorganic minerals, but several important organic dyes were also known. These included the crimson pigment, kermesic acid, the blue dye, indigo, and the yellow saffron pigment, crocetin. A rare dibromo-indigo derivative, punicin, was used to color the robes of the royal and wealthy. The deep orange hydrocarbon carotene is widely distributed in plants but is not sufficiently stable to be used as permanent pigment, other than for food

colouring. A common feature of all these coloured compounds is a system of extensively conjugated π-electrons.

Transmittance and Absorbance

As light passes through a sample, its power decreases as some of it is absorbed. This attenuation of radiation is described quantitatively by two separate, but related terms: transmittance and absorbance. Transmittance is the ratio of the source radiation's power as it exits the sample, P_T, to that incident on the sample, P_0 (Figure 29.11d.). Multiplying the transmittance by 100 gives the percent transmittance, $\%T$, which varies between 100% (no absorption) and 0% (complete absorption). All methods of detecting photons—including the human eye and modern photoelectric transducers—measure the transmittance of electromagnetic radiation. In addition to absorption by the analyte, several additional phenomena contribute to the attenuation of radiation, including reflection and absorption by the sample's container, absorption by other components in the sample's matrix, and the scattering of radiation. To compensate for this loss of the radiation's power, we use a method blank.

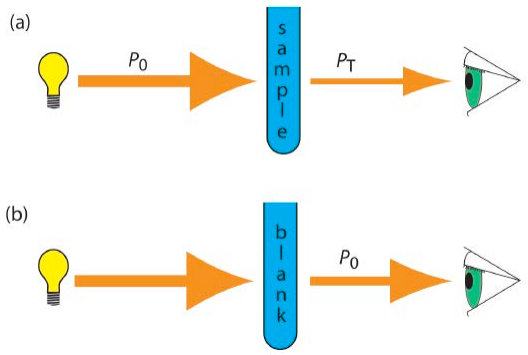

Figure 29.11d. (a) Schematic diagram showing the attenuation of radiation passing through a sample; P_0 is the source's radiant power and P_T is the radiant power transmitted by the sample. (b) Schematic diagram showing how we redefine P_0 as the radiant power transmitted by the blank. Redefining P_0 in this way corrects the transmittance in (a) for the loss of radiation due to scattering, reflection, absorption by the sample's container, and absorption by the sample's matrix. (Credit: *Instrumental Analysis*, CC BY-NC-SA 4.0)

When monochromatic (single wavelength) electromagnetic radiation passes through a thin layer of sample of thickness, it experiences a decrease in its power. This fractional decrease in power is proportional to the sample's thickness and to the analyte's concentration, *C,* in M or moles/L. The Beer-Lambert Law is:

$$\mathbf{A = \varepsilon bC}$$

where A is the absorbance, ε the molar absorptivity with units of cm^{-1} M^{-1} and b is the pathlength of the sample cell in cm. The molar absorptivity is depended on the wavelength of the absorbed photon. As such,

there is a linear relationship between absorbance and concentration, resulting in calibration curves being used in quantitative analysis.

UV-Visible Absorption

To understand why some compounds are coloured and others are not, and to determine the relationship of conjugation to colour, we must make accurate measurements of light absorption at different wavelengths in and near the visible part of the spectrum. Commercial optical spectrometers enable such experiments to be conducted with ease, and usually survey both the near ultraviolet and visible portions of the spectrum. The visible region of the spectrum comprises photon energies of 36 to 72 kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole. Ultraviolet radiation having wavelengths less than 200 nm is difficult to handle and is seldom used as a routine tool for structural analysis.

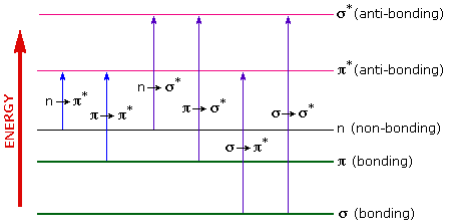

Figure 29.11e. Possible molecular electron excitations in organic molecules to a higher energy orbital from visible or UV radiation. (Credit: *Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)

The energies in the visible and ultra-violet regions are sufficient to promote or excite a molecular electron to a higher energy orbital as seen in Figure 29.11e. Of the six transitions outlined, only the two lowest energy ones (left-most, coloured blue) are achieved by the energies available in the 200 to 800 nm spectrum. As a rule, energetically favoured electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), and the resulting species is called an excited state. Consequently, absorption spectroscopy carried out in this region is sometimes called "electronic spectroscopy".

When sample molecules are exposed to light having an energy that matches a possible electronic transition within the molecule, some of the light energy will be absorbed as the electron is promoted to a higher energy orbital. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength. The resulting spectrum is presented as a graph of absorbance (A) versus wavelength. If a compound is colourless, it does not absorb in the visible part of the spectrum and this region is not displayed on the graph. Absorbance usually ranges from 0 (no absorption) to 2 (99% absorption) and is precisely defined in context with spectrometer operation.

Many electronic transitions of smaller molecules such as hydrogen or ethene are too energetic to be accurately recorded by standard UV spectrophotometers, which generally have a range of 220 – 700 nm. Where UV-vis spectroscopy becomes useful to most organic and biological chemists is in the study of molecules with conjugated pi systems. In these groups, the energy gap for π -π^* transitions is smaller than for isolated double bonds, and thus the wavelength absorbed is longer. Molecules or parts of molecules that absorb light strongly in the UV-vis region are called chromophores.

1,3-butadiene (Figure 29.11f.) is the simplest example of a system of **conjugated pi bonds**. To be considered conjugated, two or more pi bonds must be separated by only one single bond – in other words, there cannot be an intervening sp^3-hybridized carbon, because this would break up the overlapping system of parallel *p* orbitals. In Figure 29.11g., the compound's C_1-C_2 and C_3-C_4 double bonds are conjugated, while the C_6-C_7 double bond is isolated from the other two pi bonds by sp^3-hybridized C_5.

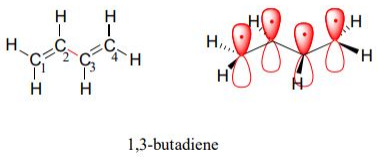

Figure 29.11f. 1,3-butadiene showing the conjugated double (pi) bond pattern (credit: *Organic Chem: Biological Emphasis Vol I (page 72)*, CC BY-NC-SA 4.0).

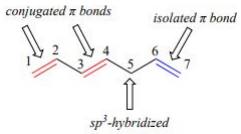

Figure 29.11g. 1,3,6-heptatriene showing conjugated and isolated pi bond sections (credit: *Organic Chem: Biological Emphasis Vol I (page 72)*, CC BY-NC-SA 4.0).

Conjugated pi systems can involve oxygen and nitrogen atoms as well as carbon. In the metabolism of fat molecules, some of the key reactions involve alkenes that are conjugated to carbonyl groups. In molecules with extended pi systems, the HOMO-LUMO energy gap becomes so small that absorption occurs in the visible rather than the UV region of the electromagnetic spectrum. Beta-carotene (Figure 29.11h.), with its system of 11 conjugated double bonds, absorbs light with wavelengths in the blue region of the visible spectrum while allowing other visible wavelengths – mainly those in the red-yellow region – to be transmitted. This is why carrots are orange.

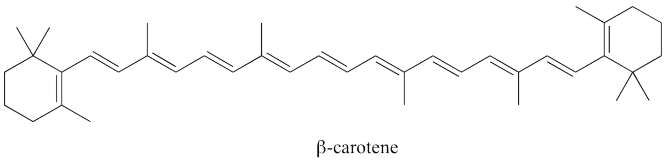

Figure 29.11h. Structure of beta-carotene with 11 conjugated double bonds (credit: *Organic Chem: Biological Emphasis Vol I (page 222)*, CC BY-NC-SA 4.0).

Example 29.11a

Identify all conjugated and isolated double bonds in the structures below.

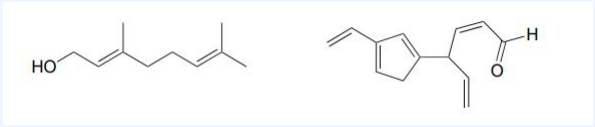

Figure 29.11i. Structure of two compounds with multiple double bonds (credit: *Organic Chem: Biological Emphasis Vol I (page 73)*, CC BY-NC-SA 4.0).

Solution:

Look for sp^3 hybridized carbons to find disruptions in conjugation.

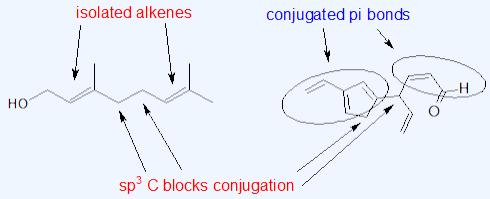

Figure 29.11j. Isolated and conjugated pi bonds in two structures with multiple double bonds. (Credit: *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0)

Source: Exercise adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

Exercise 29.11a

Identify all isolated and conjugated pi bonds in lycopene, the red-coloured compound in tomatoes.

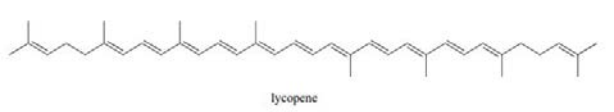

Figure 29.11k. Structure of lycopene (credit: *Organic Chem: Biological Emphasis Vol I (page 73)*, CC BY-NC-SA 4.0).

Check Your Answer:[1]

Source: Exercise and images in solution adapted from *Map: Organic Chemistry (Wade)*, CC BY-NC-SA 4.0.

UV-Vis Spectra

The basic setup for a UV-vis absorbance spectrophotometer is the same as for IR spectroscopy: radiation with a range of wavelengths is directed through a sample of interest, and a detector records which wavelengths were absorbed and to what extent the absorption occurred.

Figure 29.11l. is the absorbance spectrum of an important biological molecule called nicotinamide adenine dinucleotide, abbreviated NAD^+ . This compound absorbs light in the UV range due to the presence of conjugated pi-bonding systems.

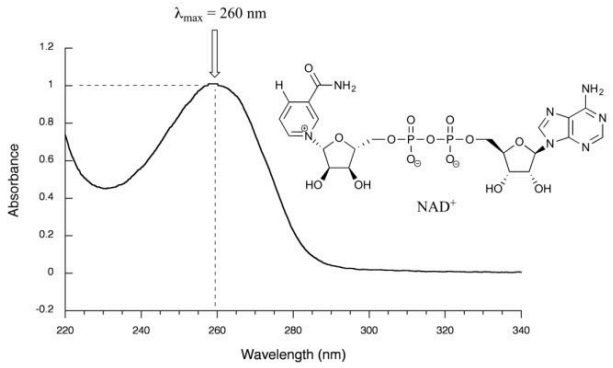

Figure 29.11l. Absorbance spectrum of nicotinamide adenine dinucleotide (NAD+) (credit: *Organic Chem: Biological Emphasis Vol I (page 224)*, CC BY-NC-SA 4.0).

This UV spectrum is much simpler than the IR spectra seen in previous sections: this one has only one peak, although many molecules have more than one. Notice also that the convention in UV-Vis spectroscopy is to show the baseline at the bottom of the graph with the peaks pointing up. Wavelength values on the x-axis are generally measured in nanometers (nm) rather than in cm^{-1} as is the convention in IR spectroscopy.

Peaks in UV spectra tend to be quite broad, often spanning well over 20 nm at half-maximal height. Typically, there are two things that are looked for and record from a UV-Vis spectrum. The first is $\lambda_{max,}$ which is the wavelength at maximal light absorbance. For NAD^+, its $\lambda_{max,}$ = 260 nm. Second is how much light is absorbed at $\lambda_{max,}$ called absorbance, abbreviated 'A' with no units. This contains the same information as the 'percent transmittance' number used in IR spectroscopy, just expressed in slightly different terms. To calculate absorbance at a given wavelength, the computer in the spectrophotometer simply takes the intensity of light at that wavelength before it passes through the sample (I_0), divides this value by the intensity of the same wavelength after it passes through the sample (I), then takes the $\log_{10}$ of that number: $A = \log I_0/I$.

Figure 29.11m. shows the absorbance spectrum of the common food colouring Red #3. Here, the extended system of conjugated pi bonds causes the molecule to absorb light in the visible range. Because the λ_{max} of 524 nm falls within the green region of the spectrum, the compound appears red to our eyes.

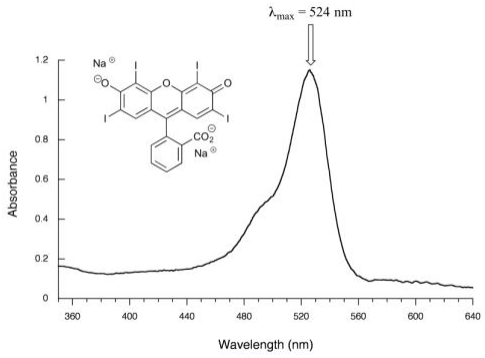

Figure 29.11m. Absorbance spectrum of the common food colouring Red #3. (credit: *Organic Chem: Biological Emphasis Vol I (page 225)*, CC BY-NC-SA 4.0).

Figure 29.11n. is the spectrum of another food colouring, Blue #1. Here, maximum absorbance is at 630 nm, in the orange range of the visible spectrum, and the compound appears blue.

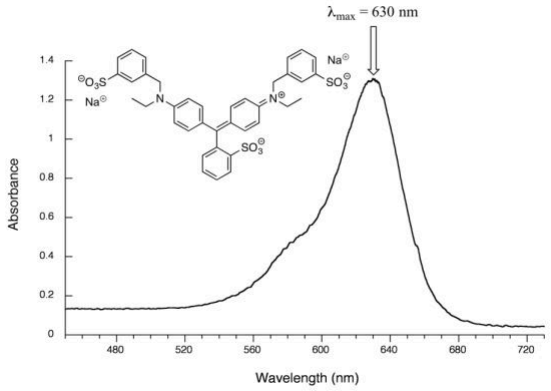

Figure 29.11n. Absorbance spectrum of the common food colouring Blue #1 (credit: *Organic Chem: Biological Emphasis Vol I (page 226)*, CC BY-NC-SA 4.0).

Exercise 29.11b

Which of the following molecules would you expect absorb at a longer wavelength in the UV region of the electromagnetic spectrum? Explain your answer.

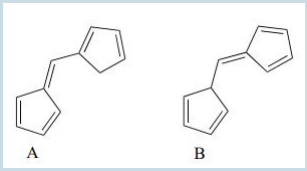

Figure 29.11o. Two structures with multiple double bonds (A and B) (credit: *Organic Chem: Biological Emphasis Vol I (page 223)*, CC BY-NC-SA 4.0).

Check Your Answer: [2]

Source: Exercise adapted from *Organic Chem: Biological Emphasis Vol I (page 222)*, CC BY-NC-SA 4.0.

Watch Conjugation & UV-Vis Spectroscopy: Crash Course Organic Chemistry #41 – YouTube (https://youtu.be/Liit4blsrqM?) (13 min).

Quantitative Applications

The determination of an analyte's concentration based on its absorption of ultraviolet or visible radiation is one of the most frequently encountered quantitative analytical methods. One reason for its popularity is that many organic and inorganic compounds have strong absorption bands in the UV/Vis region of the electromagnetic spectrum. In addition, if an analyte does not absorb UV/Vis radiation—or if its absorbance is too weak— it often can be reacted with another species that is strongly absorbing. For example, a dilute solution of Fe^{2+} does not absorb visible light. Reacting Fe^{2+} with *o*-phenanthroline, however, forms an orange–red complex of $Fe(phen)_3^{2+}$ that has a strong, broad absorbance band near 500 nm. An additional advantage to UV/Vis absorption is that in most cases it is relatively easy to adjust experimental and instrumental conditions so that Beer's law is obeyed.

The analysis of waters and wastewaters often relies on the absorption of ultraviolet and visible radiation. Aluminum, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, zinc, ammonia, cyanide, fluoride, chlorine, nitrate, nitrate, phosphate and many more can be measured using UV-Vis spectroscopy. Refer to the procedure manual of your model of spectrophotometer. For example, this site lists the Methods/Procedures for the HACH DR3900 Laboratory VIS Spectrophotometer.

Spotlight on Everyday Chemistry: Drinking Water Disinfection

Figure 29.11p. Glass of water. (credit: Photo by Aman Sachan, CC BY-SA 4.0)

Chlorine is used to disinfect and treat most drinking water at water treatment plants throughout Canada. This is used to treat the water at the source/treatment plant as well as maintain a chlorine residual in the distribution system to prevent bacterial growth. When chlorine is added to water the portion available for disinfection is called the chlorine residual (Government of Canada, 2016, para. 2).

There are two forms of chlorine residual. The free chlorine residual includes Cl_2, HOCl, and OCl^-. The

combined chlorine residual, which forms from the reaction of NH_3 with HOCl, consists of monochloramine, NH_2Cl, dichloramine, $NHCl_2$, and trichloramine, NCl_3. Because the free chlorine residual is more efficient as a disinfectant, there is an interest in methods that can distinguish between the total chlorine residual's different forms.

One such method is the leuco crystal violet method. The free residual chlorine is determined by adding leuco crystal violet to the sample, which instantaneously oxidizes to give a blue-coloured compound that is monitored at 592 nm. Completing the analysis in less than five minutes prevents a possible interference from the combined chlorine residual. The total chlorine residual (free + combined) is determined by reacting a separate sample with iodide, which reacts with both chlorine residuals to form HOI. When the reaction is complete, leuco crystal violet is added and oxidized by HOI, giving the same blue-coloured product. The combined chlorine residual is determined by difference.

Read more about the disinfection of drinking water in Ontario at Ontario.ca (https://www.ontario.ca/page/procedure-disinfection-drinking-water-ontario) and the Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Chlorine – Canada.ca (https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-chlorine-guideline-technical-document/page-2-guidelines-canadian-drinking-water-quality-chlorine-guideline-technical-document.html)

To determine the concentration of an analyte, a UV-Vis spectrophotometer is used to measure its absorbance and Beer's law is applied using a normal calibration curve using external standards. This means samples of known concentration are measured to plot a calibration curve and the unknown sample can be estimated using that curve.

Example 29.11b

The determination of iron in an industrial waste stream is carried out by the o-phenanthroline method. Using the data in the following table, determine the mg Fe/L in the waste stream.

mg Fe/L	absorbance
0.00	0.000
1.00	0.183
2.00	0.364
3.00	0.546
4.00	0.727
sample	0.269

Solution:

Linear regression (using a spreadsheet) of absorbance versus the concentration of Fe in the standards gives the calibration curve and calibration equation shown.

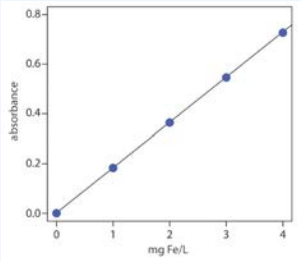

Figure 29.11q. Graph of absorbance vs mg Fe/L for experimental data. (Credit: *Instrumental Analysis*, CC BY-NC-SA 4.0)

$A = 0.0006 + (0.1817\ mg^{-1}\ L) \times (mg\ Fe/L)$

Formula does not parse

Substituting the sample's absorbance into the calibration equation gives the concentration of Fe in the waste stream as 1.48 mg Fe/L. Alternatively, the curve can be used to estimate about 1.5 mg Fe/L.

Source: Example adapted from Credit: *Instrumental Analysis*, CC BY-NC-SA 4.0.

Attribution & References

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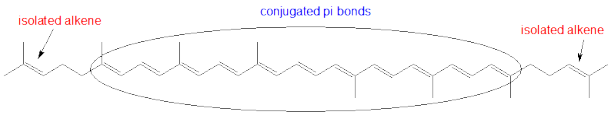
Government of Canada. (2016, January 1). *Guidelines for Canadian drinking water quality: Guideline technical document – Chlorine (https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-chlorine-guideline-technical-document.html).*

Notes

1. Here are 11 conjugated pi bonds and 2 isolated pi bonds.



2. Molecule A has a longer system of conjugated pi bonds, and thus will absorb at a longer wavelength. Notice that there is an sp3-hybridized carbon in molecule B which isolates two of the pi bonds from the other three.

CHAPTER 29 - SUMMARY

29.1 Chromatography Basics

Chromatography is an efficient way for chemists to separate and analyze mixtures. It is a method by which a mixture is separated by distributing its components between two phases. The stationary phase remains fixed in place while the mobile phase carries the components of the mixture through the medium being used. The stationary phase acts as a constraint on many of the components in a mixture, slowing them down to move slower than the mobile phase. The movement of the components in the mobile phase is controlled by the significance of their interactions with the mobile and/or stationary phases. Because of the differences in factors such as the solubility of certain components in the mobile phase and the strength of their affinities for the stationary phase, some components will move faster than others, thus facilitating the separation of the components within that mixture.

29.2 Thin Layer (TLC) and Paper Chromatography (PC)

In paper chromatography (PC), samples are separated using paper as the stationary phase. The mobile phase is typically water. The procedure for using paper chromatography is described and can be completed with household items. Paper chromatography is a visible qualitative separation method that can be quantified using R_f values (comparison of distance component travelled compared to the distance the solvent travelled). Two-way paper chromatography helps to separate components that have similar R_f values. R_f values remain consistent under consistent conditions but changing temperature or solvent will change the values.

Thin layer chromatography (TLC) is a similar technique that uses a thin layer of silica gel or alumina on a rigid surface as the stationary phase. It also involves a mobile phase of liquid solvent. In cases where components are not visible, UV fluorescence can be used to "see" the spots. This requires TLC plates that include a UV compound.

29.3 Chromatographic Columns

Chromatography is an analytical technique that separates components in a mixture. The same principles used in thin layer chromatography can be applied on a larger scale to separate mixtures in column chromatography. Column chromatography is often used to purify compounds made in the lab. Chromatographic columns are part of the instrumentation that is used in chromatography. Five chromatographic methods that use columns

are gas chromatography (GC), liquid chromatography (LC), Ion exchange chromatography (IEC), size exclusion chromatography (SEC), and chiral chromatography. The basic principles of chromatography can be applied to all five methods.

29.4 Chromatography Technology

Gas chromatography is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase. In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas that serves to carry the molecules of the analyte through the heated column.

High Performance Liquid Chromatography (HPLC) is a powerful analytical technique used for the separation of compounds soluble in a particular solvent. This separation occurs based on the interactions of the sample with the mobile and stationary phases. Because there are many stationary/mobile phase combinations that can be employed when separating a mixture, there are several different types of chromatography that are classified based on the physical states of those phases.

29.5 Spectroscopy Basics

Spectroscopy is an experimental method used by chemists to elucidate structural information. The interaction between a compound or sample and a selected region of the electromagnetic spectrum can be measured both qualitatively and quantitatively. Absorption of an appropriate quantity of energy can raise the atoms and bonds of molecules from a lower to a higher energy level, while emission of electromagnetic radiation corresponds to a change from a higher to a lower energy level. The resulting spectrum can be used to determine structural information about the molecule.

29.6 Infrared (IR) Spectroscopy

The infrared region of the electromagnetic spectrum causes asymmetric bonds to stretch, bend, and/or vibrate. This interaction can be measured to help elucidate chemical structures. Asymmetry and polarity increase the strength of IR absorption (infrared active). Symmetrical carbon-carbon double and triple bonds will not absorb IR light and are called “infrared inactive”. The analysis and interpretation of the IR spectra for several compounds are explained. One of the most common applications of infrared spectroscopy is the identification of organic compounds. The IR spectra for the major classes of organic molecules are shown and can be used to help determine present functional groups. Numerous examples are provided.

29.7 Mass Spectrometry (MS)

Mass spectrometry is an analytic method that employs ionization and mass analysis of compounds in order to determine the mass, formula and structure of the compound being analyzed. A mass spectrometer creates charged particles (ions) from molecules. It then analyzes those ions to provide information about the molecular weight of the compound and its chemical structure. A mass analyzer is the component of the mass spectrometer that takes ionized masses and separates them based on charge to mass ratios and outputs them to the detector where they are detected and later converted to a digital output. Fragmentation patterns are formed when organic molecules are fed into a mass spectrometer. When interpreting fragmentation patterns, the weakest carbon-carbon bonds are the ones most likely to break. The relative formula mass (relative molecular mass) of an organic compound can be determined from its mass spectrum. Mass spectrometry is widely used in industry through the coupling Gas Chromatography (GC) with Mass Spectrometry (MS).

29.8 Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) Spectroscopy uses the electromagnetic radiation of radio waves to probe the local electronic interactions of a nucleus. The chemical shift is the resonant frequency of a nucleus relative to a standard in a magnetic field (often TMS). The position and number of chemical shifts provide structural information about a molecule.

29.9 ^{1}H NMR Spectroscopy

Proton NMR finds use for both qualitative analyses and quantitative analyses. In an applied, external magnetic field, protons in different locations of a molecule have different resonance frequencies, because they are in non-identical electronic environments. Equivalent protons experience the same electronic environment. An approximate idea of the chemical shifts of the most common types of protons is helpful when interpreting ^{1}H NMR spectra. Tables of values are available to help with determining the molecular location of protons. The ratio of proton signal areas correlates with the proton ratio of a compound providing useful structural information. The peaks can be split into multiplets when the magnetic field experienced by the protons of one group is influenced by the spin arrangements of the protons in an adjacent group. Splitting occurs primarily between non-equivalent hydrogens that are separated by three bonds.

29.10 ^{13}C NMR Spectroscopy

The ^{12}C isotope of carbon – which accounts for up about 99% of the carbons in organic molecules – does not have a nuclear magnetic moment, and thus is NMR-inactive. Fortunately for organic chemists, however,

the ^{13}C isotope, which accounts for most of the remaining 1% of carbon atoms in nature, has a magnetic moment just like protons. Most of what we have learned about ^{1}H-NMR spectroscopy also applies to ^{13}C-NMR, although there are several important differences. ^{13}C chemical shifts are analogous to proton chemical shifts and are influenced by the electro-magnetic environment of the carbon atoms. Tables of values are available for chemical shifts of carbon atoms and can be useful in determining the carbon skeletal structure of molecules.

29.11 Visible and Ultra-Violet Spectroscopy (UV-Vis)

As light passes through a sample, its power decreases as some of it is absorbed. This attenuation of radiation is described quantitatively by two separate, but related terms: transmittance and absorbance. Beer's law connects absorbance to the concentration of the absorbing species. The determination of an analyte's concentration based on its absorption of ultraviolet or visible radiation is one of the most common quantitative analytical methods. In addition, if an analyte does not absorb UV/Vis radiation—or if its absorbance is too weak—we often can react it with another species that is strongly absorbing.

The conjugated double bonds are characterized by alternating carbon-carbon bonds separated by carbon-carbon single bonds. The stability of conjugated dienes can be explained using the delocalization of charge through resonance. The ultraviolet absorption maximum of a conjugated molecule is dependent upon the extent of conjugation. The ultraviolet (UV) region of the electromagnetic spectrum corresponds to conjugated bond energies. The most useful UV region of the electromagnetic spectrum has a wavelength between 200 and 400 nm. Absorption in the visible region of the electromagnetic spectrum results in coloured compounds.

Attribution & References

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- 29.9 Summary adapted from "19: Nuclear Magnetic Resonance Spectroscopy" by David Harvey In *Instrumental Analysis*, CC BY-NC-SA 4.0 and "12: Nuclear Magnetic Resonance Spectroscopy"In *Map: Organic Chemistry (Wade), Complete and Semesters I and II* , CC BY-NC-SA 4.0.
- 29.10 Summary adapted from "12: Nuclear Magnetic Resonance Spectroscopy" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II* , CC BY-NC-SA 4.0.
- 29.11 Summary adapted from "13: Introduction to Ultraviolet/Visible Absorption Spectrometry" and "14: Applications of Ultraviolet/Visible Molecular Absorption Spectrometry" by David Harvey In *Instrumental Analysis*, CC BY-NC-SA 4.0 and "16: Conjugated Systems, Orbital Symmetry, and Ultraviolet Spectroscopy" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II* , CC BY-NC-SA 4.0.

CHAPTER 29 - REVIEW

29.1 Chromatography Basics

1. What is the purpose of chromatography? **Check answer**[1]
2. Describe the purpose of the stationary phase and the mobile phase. **Check answer**[2]
3. Why do substances travel at different rates? **Check answer**[3]

29.2 Thin Layer (TLC) and Paper Chromatography (PC)

1. Try your own paper chromatography using household items. Stationary phase – paper towel or coffee filter, Mobile phase – water or rubbing alcohol, Mixture – water soluble markers, water soluble wet paint, food colouring. Describe the results and how you might adjust the experiment to get different results.
2. Calculate the R_f values for the following separation: Solvent travelled 5.8 cm, Compound A travelled 2.1 cm, Compound B travelled 2.3 cm, Compound C travelled 4.0 cm, and Compound D travelled 5.6 cm. What conclusions can you make about Compounds A through D? **Check answer**[4]
3. Describe some of the factors that will influence a component's R_f value.

29.3 Chromatographic Columns

1. Research some uses of ion exchange chromatographic (IEC) columns and size exclusion chromatographic (SEC) columns.
2. What do all types of chromatographic columns have in common? **Check answer**[5]
3. What are some benefits of chromatographic columns over paper chromatography or thin-layer chromatography? **Check answer**[6]

29.4 Chromatography Technology

1. Explain the principles of gas chromatography (GC).
2. Explain the principles of high-performance liquid chromatography (HPLC).
3. Consider what a scientist may need to consider when choosing a chromatography method. Complete this chart. Research may be needed.

Chart 1: What to consider when choosing a chromatography method. Columns are left blank for you to fill in.

Chromatography Method	PC/ TLC	IEC	GC	HPLC
Can the method be used to physically separate sample into different containers?				
Can the method be used to check purity of sample?				
Can the method be used in combination with another method to determine compound identity?				
What type of samples are required? Consider states, amount of sample, polarities, charges.				
Is the method inexpensive or expensive to run?				
Is the method readily available or does it require specialized lab equipment?				
How long does the method take to get a result? (quick or slow)				

Links to Enhanced Learning

For more questions about Chromatography, try Separation of solutions and mixtures

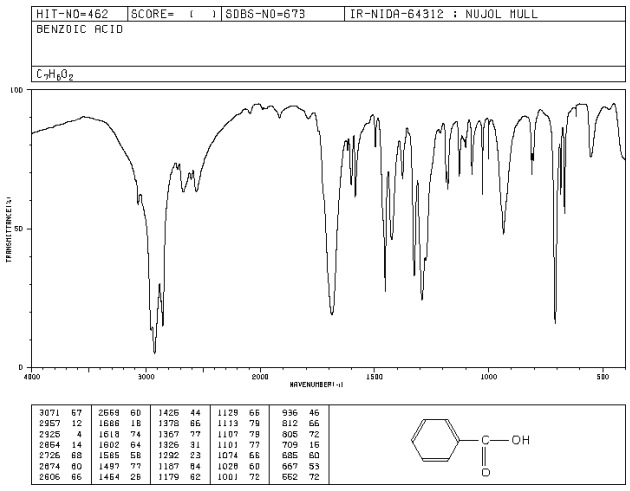
chromatography (practice) [New tab] (https://www.khanacademy.org/science/ap-chemistry-beta/x2eef969c74e0d802:intermolecular-forces-and-properties/x2eef969c74e0d802:separation-of-solutions-and-mixtures-chromatography/e/separation-of-solutions-and-mixtures-chromatography)

29.5 Spectroscopy Basics

1. What is the purpose of spectroscopy? **Check answer**[7]
2. What is a spectrum? **Check answer**[8]

29.6 Infrared (IR) Spectroscopy

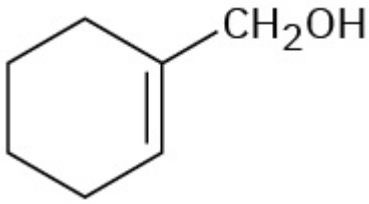
1. What functional groups give the following signals in an IR spectrum?
   a. 1700 cm^{-1}
   b. 1550 cm^{-1}
   c. 1700 cm^{-1} and 2510-3000 cm^{-1} **Check answer**[9]
2. How can you distinguish the following pairs of compounds through IR analysis?
   a. CH_3OH (Methanol) and $CH_3CH_2OCH_2CH_3$ (Diethylether)
   b. cyclopentane and 1-pentene.
   c. 1-propanol and 2-propanol. **Check answer**[10]
3. The following spectrum is for the accompanying compound, benzoic acid. What are the peaks that you can identify in the spectrum? **Check answer**[11]



(**Credit:** "Benzoic acid – compound 673" © National Institute of Advanced Industrial Science and Technology via SDBSWeb , accessed 11/21/2023.)

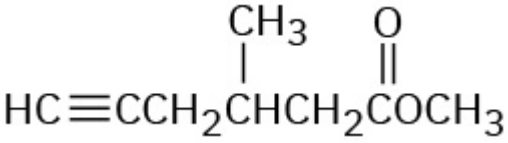
4. Using the video IR spectra practice | Spectroscopy | Organic chemistry | Khan Academy – YouTube (https://www.youtube.com/watch?v=mJt1QI-yDG4&t=16s), predict which molecule has the shown IR spectrum. Spectrum 1 – stop the video at 0:16. Determine which of the three molecules is the correct one. Watch the video for an explanation of the answer. Spectrum 2 – starts at 2:00, stop at 2:05. Determine which of the three molecules is the correct one. Watch the video for an explanation of the answer. Spectrum 3 – starts at 3:34, stop at 3:42. Determine which of the three molecules is the correct one. Watch the video for an explanation of the answer.
5. Where might the following compounds have IR absorptions? **Check answer**[12]

   a)



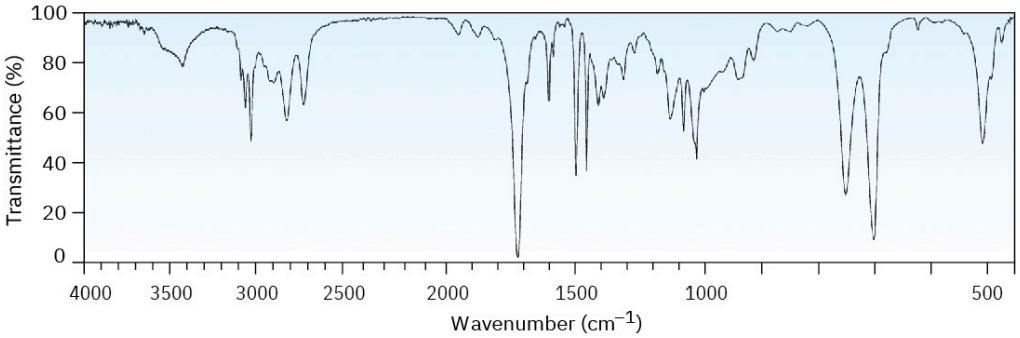
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

   b)



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

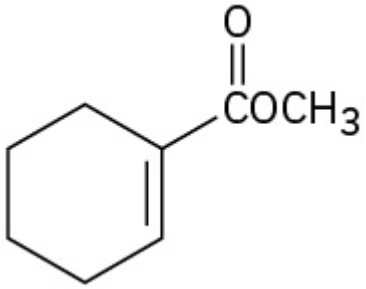
6. The IR spectrum of an unknown compound is shown. What functional groups does the compound contain? **Check answer**[13]



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

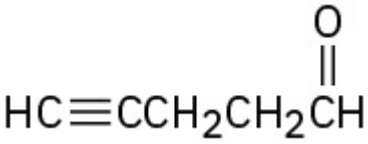
7. Where might the following compounds have IR absorptions? **Check answer**[14]

   a)



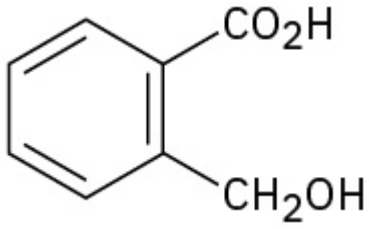
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

b)



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

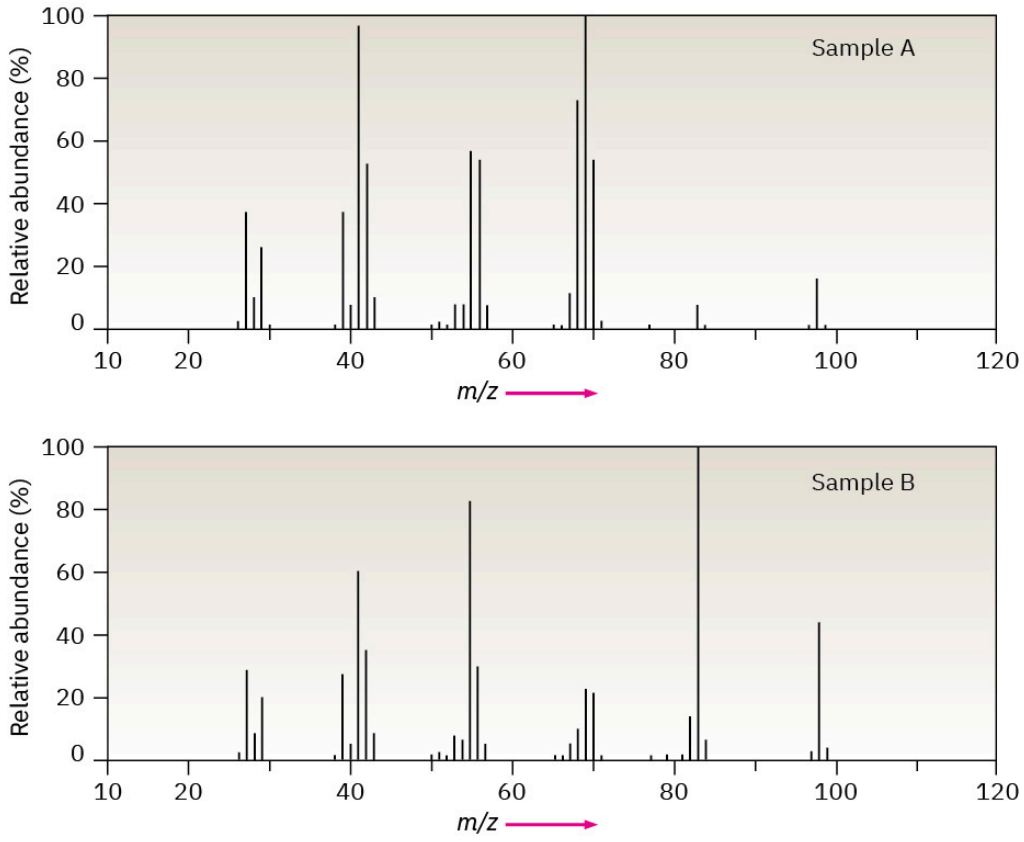
c)



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

29.7 Mass Spectrometry (MS)

1. What are three things that can be determined using MS? **Check answer**[15]
2. Assume that you have two unlabeled samples, one of methylcyclohexane and the other of ethylcyclopentane. How could you use mass spectrometry to tell them apart? The mass spectra of both are shown. **Check answer**[16]



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

3. The sex hormone testosterone contains only C, H, and O and has a mass of 288.2089 amu, as determined by high-resolution mass spectrometry. What is the likely molecular formula of testosterone? **Check answer**[17]

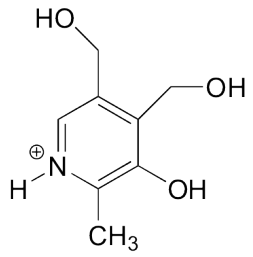
29.8 Nuclear Magnetic Resonance (NMR)

1. What is a chemical shift? **Check answer**[18]
2. What effect does the magnetic field have on atoms? **Check answer**[19]

29.9 ^{1}H NMR Spectroscopy

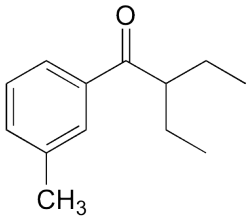
1. How many different sets of protons do the following molecules contain?

a)



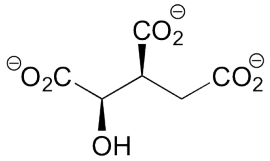
pyridoxine
(vitamin B_6)

b)



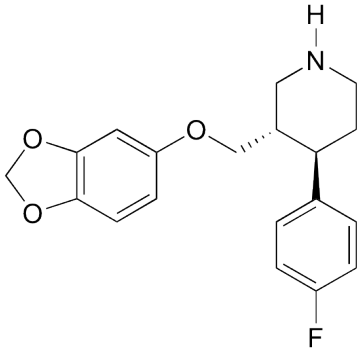
DEET
(an insect repellent)

c)



isocitrate
(a citric acid cycle intermediate)

d)

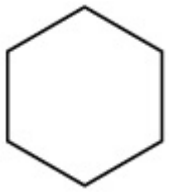


paroxetine
(an antianxietry drug, trade name Paxil)

(Credit: *Map: Organic Chemistry (Wade), Complete and Semesters I and II*, CC BY-NC-SA 4.0).

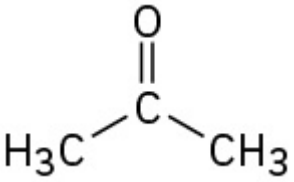
2. How many non-equivalent hydrogens are in the following molecules? How many different signals will you see in a H^1 NMR spectrum? **Check answer**[20]
    a. $CH_3CH_2CH_2Br$
    b. $CH_3OCH_2C(CH_3)_3$
    c. Ethyl Benzene
    d. 2-methyl-1-hexene
3. Methyl 2,2-dimethylpropanoate $(CH_3)_2CCO_2CH_3$ has two peaks in its ^1H NMR spectrum. What are their approximate chemical shifts? **Check answer**[21]
4. Each of the following compounds has a single ^1H NMR peak. Approximately where would you expect each compound to absorb? **Check answer**[22]

    a)



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

    b)

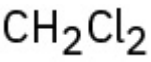


(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

c)

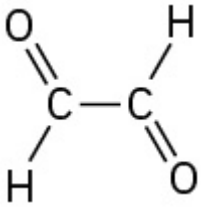
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

d)



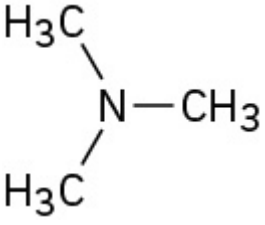
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

e)



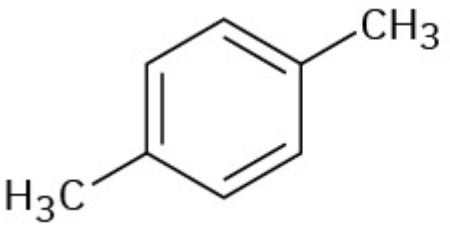
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

f)



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

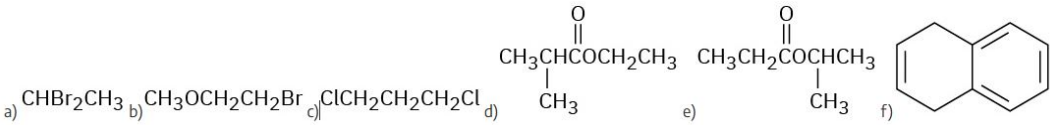
5. How many peaks would you expect in the ^{1}H NMR spectrum of 1,4-dimethylbenzene (*para*-xylene, or *p*-xylene)? What ratio of peak areas would you expect on integration of the spectrum? **Check answer**[23]



p-Xylene

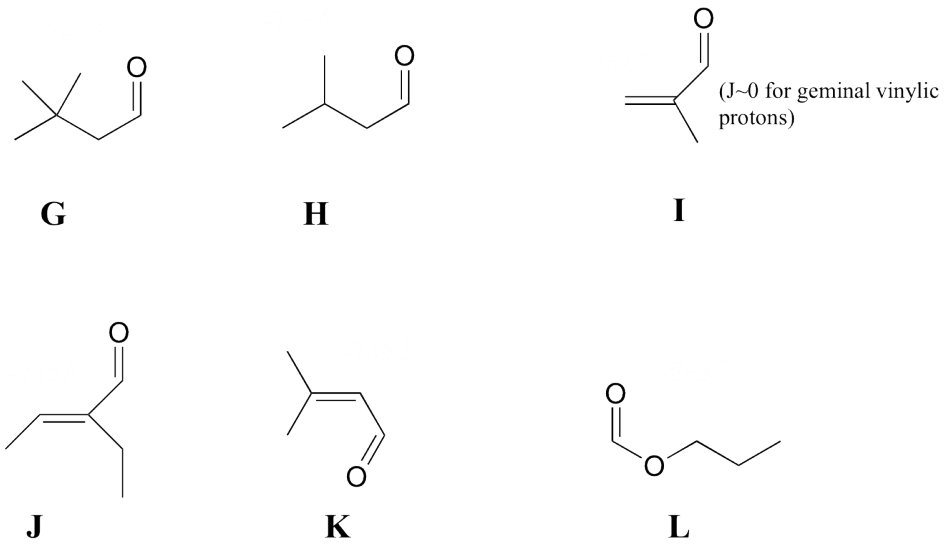
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

6. Predict the splitting patterns you would expect for each proton in the following molecules: **Check answer**[24]



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

7. Match spectra 7-12 below to their corresponding structures G-L.



(Credit: *Map: Organic Chemistry (Wade), Complete and Semesters I and II*, CC BY-NC-SA 4.0).

Spectrum 7 table

	δ	splitting	integration
9.96		d	1
5.88		d	1
2.17		s	3
1.98		s	3

Spectrum 8 table

δ	splitting	integration
9.36	s	1
6.55	q	1
2.26	q	2
1.99	d	3
0.96	t	3

Spectrum 9 table

δ	splitting	integration
9.57	s	1
6.30	s	1
6.00	s	1
1.84	s	3

Spectrum 10 table

δ	splitting	integration
9.83	t	1
2.27	d	2
1.07	s	9

Spectrum 11 table

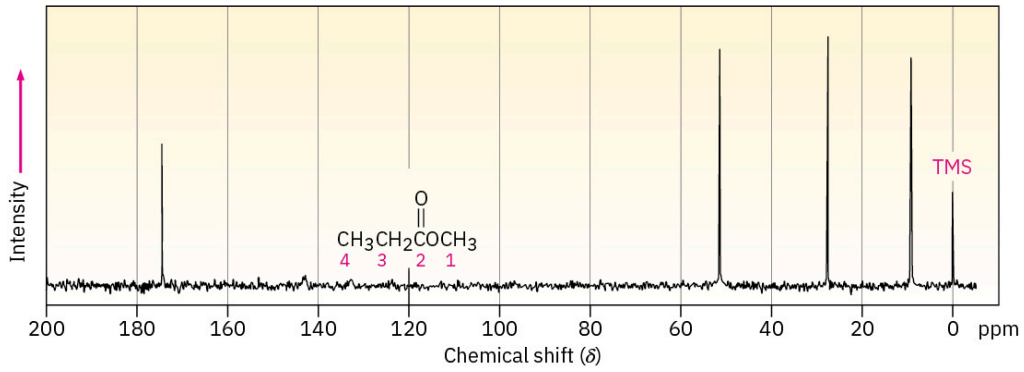
δ	splitting	integration
9.75	t	1
2.30	dd	2
2.21	m	1
0.98	d	6

Spectrum 12 table

δ	splitting	integration
8.08	s	1
4.13	t	2
1.70	m	2
0.96	t	3

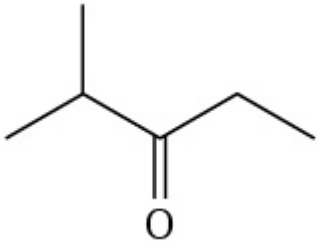
29.10 ^{13}C NMR Spectroscopy

1. At what approximate positions would you expect ethyl acrylate, $H_2C{=}CHCO_2CH_2CH_3$, to show ^{13}C NMR absorptions? **Check answer**[25]
2. Classify the resonances in the ^{13}C NMR spectrum of methyl propanoate, $CH_3CH_2CO_2CH_3$. **Check answer**[26]



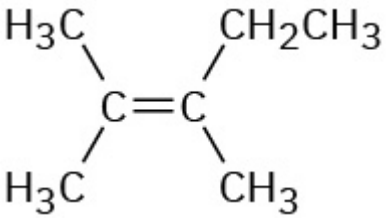
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

3. Predict the number of carbon resonance lines you would expect in the ^{13}C NMR spectra of the following compounds: **Check answer**[27] a) Methylcyclopentane b) 1-Methylcyclohexene c) 1,2-Dimethylbenzene d) 2-Methyl-2-butene e)



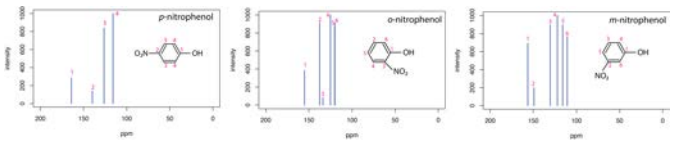
(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

f)



(credit: *Organic Chemistry (OpenStax)*, CC BY-NC-SA 4.0)

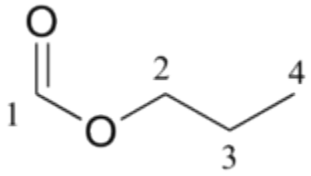
4. This figure shows ^{13}C NMR spectrum for three related molecules: *p*-nitrophenol, *o*-nitrophenol, and *m*-nitrophenol. Identify some ^{13}C NMR differences between these isomers. Try to explain the differences using the molecule structure.



(Credit: Original data used to construct these spectra is © National Institute of Advanced Industrial Science and Technology via SDBSWeb. The spectra were recorded on a 15 MHz instrument (with respect to 13C, or 60 MHz with respect to 1H). Credit:*Instrumental Analysis*, CC BY-NC-SA 4.0)

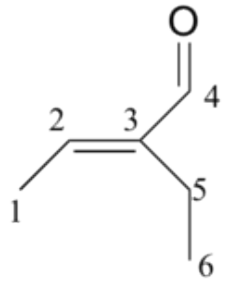
5. ^{13}C-NMR data is given for the molecules shown below. Complete the peak assignment column of each NMR data table.

Table A



δ	carbon fragment	carbon #
161.12	CH	
65.54	CH_2	
21.98	CH_2	
10.31	CH_3	

Table B



δ	carbon fragment	carbon #
194.72	C	
149.10	C	
146.33	CH	
16.93	CH_2	
14.47	CH_3	
12.93	CH_3	

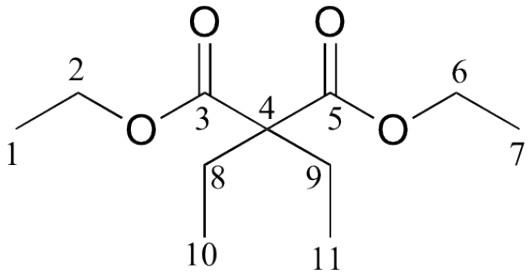


Table C

δ	carbon fragment	carbon #
171.76	C	
60.87	CH_2	
58.36	C	
24.66	CH_2	
14.14	CH_3	
8.35	CH_3	

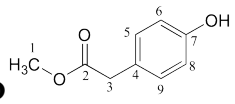


Table D

δ	carbon fragment	carbon #
173.45	C	
155.01	C	
130.34	CH	
125.34	C	
115.56	CH	
52.27	CH_3	
40.27	CH_2	

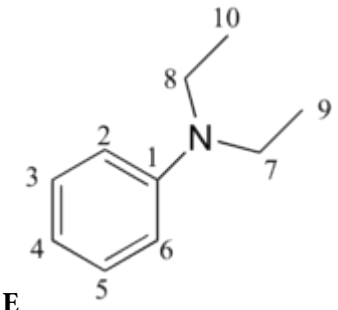


Table E

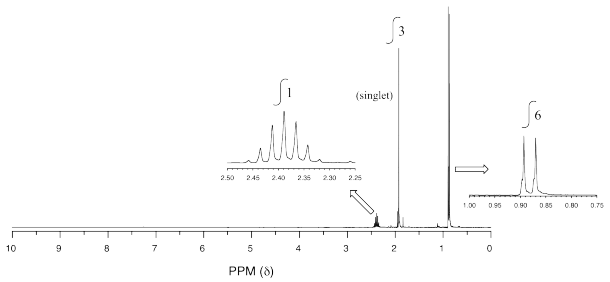
δ	carbon fragment	carbon #
147.79	C	
129.18	CH	
115.36	CH	
111.89	CH	
44.29	CH_2	
12.57	CH_3	

Image Credit: *Organic Chemistry with a Biological Emphasis Volume I, (page 296-297)* CC BY-NC-SA 4.0

6. Combining NMR and MS. You obtain the following data for an unknown sample. Deduce its structure.

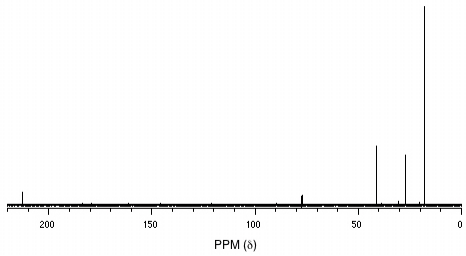
Combustion Analysis: C (69.7%), H (11.7%)

^{1}H-NMR:



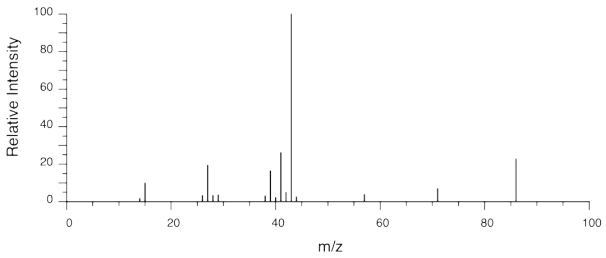
(*Credit:* *Organic Chemistry with a Biological Emphasis Volume I*, *(page 301)* CC BY-NC-SA 4.0)

^{13}C-NMR:



(*Credit:* *Organic Chemistry with a Biological Emphasis Volume I*, *(page 301)* CC BY-NC-SA 4.0)

Mass Spectrometry:



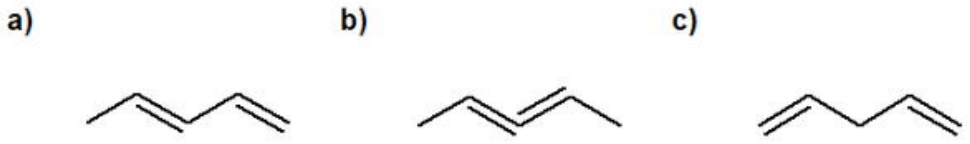
(*Credit:* *Organic Chemistry with a Biological Emphasis Volume I*, *(page 300)* CC BY-NC-SA 4.0)

Links to Enhanced Learning

- Spectroscopy | Organic chemistry | Science | Khan Academy [New tab] (https://www.khanacademy.org/science/organic-chemistry/spectroscopy-jay) has some videos where IR and NMR problems are presented and solved.
- The Chemical Detectives app on Chemical Detectives – Apps on Google Play [New tab] (https://play.google.com/store/apps/details?id=com.chemicaldetectives&pli=1) and Chemical Detectives on the App Store [New tab] (https://apps.apple.com/au/app/chemical-detectives/id741760895) allows users to browse various types of spectra (IR, MS, ^{1}H NMR, ^{13}C NMR, and elemental microanalysis) for simple organic compounds and also complete quizzes about various compounds based on their spectra. Customize of types of functional groups is possible (e.g. only hydrocarbons or only alcohols).
- Predict 1H proton NMR spectra [New tab] (https://www.nmrdb.org/new_predictor/index.shtml?v=v2.138.0) offers opportunities to predict ^{1}H and ^{13}C NMR.

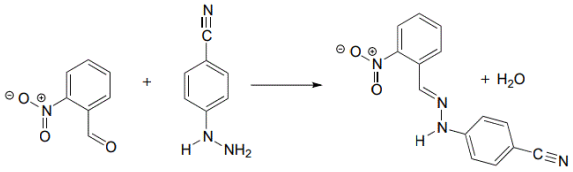
29.11 Visible and Ultra-Violet Spectroscopy (UV-Vis)

1. What is a conjugated double bond? **Check answer**[28]
2. Identify which of the following dienes are isolated, conjugated, or cumulated. **Check answer**[29]



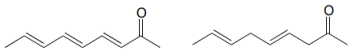
(Credit: *Map: Organic Chemistry (Wade), Complete and Semesters I and II*, CC BY-NC-SA 4.0).

3. A solution of analyte, with molar absorptivity of 676 cm^{-1} M^{-1}, is placed in a sample cell that has a pathlength of 1.00 cm. At a wavelength of 490 nm, the solution's absorbance is 0.228. What is the analyte's concentration? **Check answer**[30]
4. A reaction shown below. While the two starting materials are only slightly coloured, the product is an intense orange-red. Account for this observation.



(*Credit: Organic Chemistry with a Biological Emphasis Volume I, (page 232)* CC BY-NC-SA 4.0)

5. Which would be more useful in distinguishing the two compounds shown below. IR or UV spectroscopy? Explain. **Check answer**[31]



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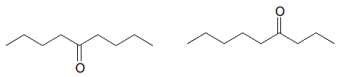
6. Which analytical technique – IR, UV, or MS – could best be used to distinguish between the two compounds below? Explain. **Check answer**[32]



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Attribution & References

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29.1 to section 29.5 review questions

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29.6 review questions

- Q1-2 from 11.5: Infrared Spectra of Some Common Functional Groups and Q5 – Q7 from 12.8 Infrared Spectra of Some Common Functional Groups In *Organic Chemistry (OpenStax)* by John McMurry, licensed under CC BY-NC-SA 4.0. Access for free at *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/12-8-infrared-spectra-of-some-common-functional-*

groups)

- 29.6 Question 6 image in solution from: *Organic Chemistry (OpenStax),* CC BY-NC-SA 4.0

29.7

- Q1 written by Samantha Sullivan Sauer, shared under CC BY-NC 4.0
- Q2-3 by "12.2 Interpreting Mass Spectra" In *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)* by John McMurray, CC BY-NC-SA 4.0. Access for free at *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)*

28.8

- Q1-2 written by Samantha Sullivan Sauer, shared under CC BY-NC 4.0

29.9

- Section 29.9, questions 1-2 from "12.4: ^{1}H NMR Spectroscopy and Proton Equivalence" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II*by LibreTexts, licensed under CC BY-NC-SA 4.0.
- Q3-4 from "13.4 Chemical Shifts in 1H NMR Spectroscopy" In *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)* by John McMurray, CC BY-NC-SA 4.0. Access for free at *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)*
- Q5 From "13.5 Integration of 1H NMR Absorptions: Proton Counting" In *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)* by John McMurray, CC BY-NC-SA 4.0. Access for free at *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)*
- Q6 from "13.6 Spin–Spin Splitting in 1H NMR Spectra" In *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)* by John McMurray, CC BY-NC-SA 4.0. Access for free at *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)*
- Q7 From "12.14: More NMR Examples" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II*, CC BY-NC-SA 4.0

29.10

- Q1, Q2 & Q3 – From "13.11 Characteristics of 13C NMR Spectroscopy" In *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)* by John McMurray,

CC BY-NC-SA 4.0. Access for free at *Organic Chemistry (OpenStax) (https://openstax.org/books/organic-chemistry/pages/1-why-this-chapter)*

- Q1 image in solution from *Organic Chemistry (OpenStax),* CC BY-NC-SA 4.0
- Q4 – From "19.5: Carbon-13 NMR" by David Harvey In *Instrumental Analysis (LibreTexts)*, CC BY-NC-SA 4.0.
- Q5 – From: "12.14: More NMR Examples" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II* , CC BY-NC-SA 4.0.
- Q6 – Modified from Organic Chemistry with a Biological Emphasis Volume I (umn.edu) (p. 299) and "12.14: More NMR Examples" In *Map: Organic Chemistry (Wade), Complete and Semesters I and II* , CC BY-NC-SA 4.0.

29.11

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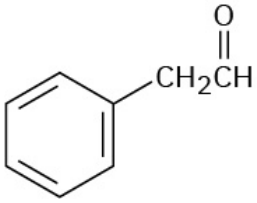
- Q3 is adapted from "13.2: Beer's Law" by David Harvey In *Instrumental Analysis*, licensed under CC BY-NC-SA 4.0
- Q4-6 are adapted from p. 232 In *Organic Chemistry with a Biological Emphasis Volume I* by Timothy Soderberg, licensed under CC BY NC SA 4.0

Notes

1. Chromatography separates a mixture into it's dissolved components.
2. The stationary phase (typically a solid) does not move and holds onto the mixture and it's components. The mobile phase moves (typically a gas or liquid) and drags the mixture's components with it at varying rates.
3. The rate of travel depends on the components affinity for the stationary phase over the mobile phase. More affinity to the stationary phase means the component will travel slower.
4. Compound A $R_f = 0.36$; Compound B $R_f = 0.40$; Compound C $R_f = 0.69$; Compound D $R_f = 0.97$. Compound D has high affinity to the mobile phase and low affinity for the stationary phase. Compounds A and B have similar affinities for the stationary phase.
5. All columns require a stationary phase and a mobile phase of different properties to separate the applied mixture.
6. Columns allow for collection of the mixture's components whereas TLC/PC are only visual and can't capture the components. PC/TLC are typically much quicker and cheaper to perform.
7. To determine the structure of a compound.
8. The pattern in which matter absorbs or emits radiation.
9. A) carbonyl group in aldehydes, ketones, carboxylic acids, amides, esters B) aromatics, amines, nitro, C) carboxylic acids

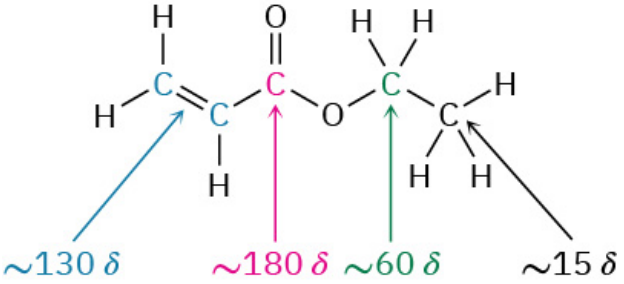
10. A) A OH peak will be present around 3300 cm^{-1} for methanol and will be absent in the ether. B) 1-pentene will have an alkene peak around 1650 cm^{-1} for the C=C and there will be another peak around 3100 cm^{-1} for the sp^2 C-H group on the alkene C) Cannot distinguish these two isomers. They both have the same functional groups and therefore would have the same peaks on an IR spectra.
11. 1680 cm^{-1} for carbonyl group in carboxylic acid, 2820 cm^{-1} for OH in carboxylic acid, and 2925 cm^{-1} for CH in aromatics.
12. Strategy: Identify the functional groups in each molecule, and then check an IR table of values. (a) Absorptions: 3400 to 3650 cm^{-1} (O–H), 3020 to 3100 cm^{-1} (=C–H), 1640 to 1680 cm^{-1} (C═C). This molecule has an alcohol O–H group and an alkene double bond. (b) Absorptions: 3300 cm^{-1} (≡C–H), 2100 to 2260 cm^{-1} (C≡C), 1735 cm^{-1} (C═O). This molecule has a terminal alkyne triple bond and a saturated ester carbonyl group.
13. Strategy: All IR spectra have many absorptions, but those useful for identifying specific functional groups are usually found in the region from 1500 cm^{-1} to 3300 cm^{-1}. Pay particular attention to the carbonyl region (1670 to 1780 cm^{-1}), the aromatic region (1660 to 2000 cm^{-1}), the triple-bond region (2000 to 2500 cm^{-1}), and the C–H region (2500 to 3500 cm^{-1}). Solution: The spectrum shows an intense absorption at 1725 cm^{-1} due to a carbonyl group (perhaps an aldehyde, –CHO), a series of weak absorptions from 1800 to 2000 cm^{-1} characteristic of aromatic compounds, and a C–H absorption near 3030 cm^{-1}, also characteristic of aromatic compounds. In fact, the compound is phenylacetaldehyde.



Phenylacetaldehyde

14. (a) 1715, 1640, 1250 cm^{-1} (b) 1730, 2100, 3300 cm^{-1} (c) 1720, 2500–3100, 3400–3650 cm^{-1}
15. mass, formula and structure of compound
16. Strategy: Look at the possible structures and decide on how they differ. Then think about how any of these differences in structure might give rise to differences in mass spectra. Methyl cyclohexane, for instance, has a $–CH_3$ group, and ethylcyclopentane has a $–CH_2CH_3$ group, which should affect the fragmentation patterns. Solution: Both mass spectra show molecular ions at $M^+ = 98$, corresponding to C_7H_{14}, but they differ in their fragmentation patterns. Sample **A** has its base peak at $m/z = 69$, corresponding to the loss of a CH_2CH_3 group (29 mass units), but **B** has a rather small peak at $m/z = 69$. Sample **B** shows a base peak at $m/z = 83$, corresponding to the loss of a CH_3 group (15 mass units), but sample **A** has only a small peak at $m/z = 83$. We can therefore be reasonably certain that **A** is ethylcyclopentane and **B** is methylcyclohexane.
17. $C_{19}H_{28}O_2$
18. The position on the plot at which the nuclei absorbs.
19. It aligns the nuclear spins of the atoms.
20. A. 3; B. 3; C. 5; D. 7
21. Strategy: Identify the types of hydrogens in the molecule, and note whether each is alkyl, vinylic, or next to an electronegative atom. Then predict where each absorbs. Solution: The $–OCH_3$ protons absorb around 3.5 to 4.0 **δ** because they are on carbon bonded to oxygen. The $(CH_3)_3C–$ protons absorb near 1.0 **δ** because they are typical alkane-like protons.
22. (a) 1.43 δ (b) 2.17 δ (c) 7.37 δ (d) 5.30 δ (e) 9.70 δ (f) 2.12 δ
23. Two peaks; 3 : 2 ratio
24. (a) $–CHBr_2$, quartet; $–CH_3$, doublet (b) $CH_3O–$, singlet; $–OCH_2–$, triplet; $–CH_2Br$, triplet (c) $ClCH_2–$, triplet; $–CH_2–$, quintet (d) $CH_3–$, triplet; $–CH_2–$, quartet; –CH– , septet; $(CH_3)_2$, doublet (e) $CH_3–$, triplet; $–CH_2–$, quartet; –CH–, septet; $(CH_3)_2$, doublet (f) =CH, triplet, $–CH_2–$, doublet, aromatic C–H, two multiplets

25. Strategy: Identify the distinct carbons in the molecule, and note whether each is alkyl, vinylic, aromatic, or in a carbonyl group. Then predict where each absorbs. Solution: Ethyl acrylate has five chemically distinct carbons: two different C=C, one C=O, one O–C, and one alkyl C. The likely absorptions are:



The actual absorptions are at 14.1, 60.5, 128.5, 130.3, and 166.0 δ.

26. $-CH_3$, 9.3 δ; $-CH_2-$, 27.6 δ; C=O, 174.6 δ;– OCH_3, 51.4 δ
27. (a) 4 (b) 7 (c) 4 (d) 5 (e) 5 (f) 7
28. conjugated double bonds are alternating carbon-carbon double bonds separated by carbon-carbon single bonds.
29. a) conjugated, b) cumulated, c) isolated
30. 3.37×10^{-4} M
31. UV because the molecule on the left has conjugation whereas the molecule on the right does not. Their IR spectra would be very similar as their functional groups are similar.
32. MS because both molecules have the same functional groups and neither have conjugation. The MS spectra will show different fragments due to the location of the carbonyl group.

CHAPTER 29 - INFOGRAPHIC DESCRIPTIONS

Infographics used in Chapter 29

- 29.6a Analytical Chemistry – Infrared (IR) Spectroscopy
- 29.7a Mass spectrometry and a guide to interpreting mass spectra
- 29.9a Analytical Chemistry – A Guide to Proton Nuclear Magnetic Resonance (NMR)
- 29.10a A Guide to 13-C Nuclear Magnetic Resonance (NMR)

29.6a Analytical Chemistry – Infrared (IR) Spectroscopy

Commonly referred to as IR spectroscopy, this technique allows chemists to identify characteristic groups of atoms (functional groups) present in molecules.

The fingerprint region: 1500cm^{-1} to 500cm^{-1}. The fingerprint region of the spectrum contains a complex set of absorptions, which are unique to each compound. Though these are hard to interpret visually, by comparison with references they allow identification of specific compounds.

Infrared frequencies make up a portion of the electromagnetic spectrum. If a range of infrared frequencies are shone through an organic compound, some of the frequencies are absorbed by the chemical bonds within the compound. Different chemical bonds absorb different frequencies of infrared radiation. There are a number of characteristic absorptions which allow functional groups (the parts of a compound which give it its particular reactivity) to be identified.

Infrared Spectroscopy – Approximate Frequencies of Bond Vibrations

Frequency (estimated) (cm^{-1})	**Functional Group**	**Bond**	**Strength of Signal**
515-690	Alkyl bromide	C-X stretch	medium
550-850	Alkyl chloride	C-X stretch	medium
600-700	Alkene	C-H bend	strong, broad
650-900	Primary and secondary amine	N-H wag	strong, broad
760-1000	Alkene	C-H bend	strong
900-950	Carboxylic acid	O-H bend	medium
1000-1250	Aliphatic amines	C-N stretch	variable
1000-1325	Esters, ethers, alcohols, carboxylic acids	C-O stretch	strong
1175-1300	Haloalkane	C-H wag	medium
1250-1350	Nitro compound	N-O symm. stretch	medium
1350-1450	Alkane	C-H bend, rock	medium
1400-1600	Aromatics	C-C stretch	medium
1450-1550	Nitro compound	N-O asymm. stretch	strong
1525-1625	Primary amine	N-H bend	medium
1550-1650	Alkene	C=C stretch	medium
1650-1750	Carbonyls (acid, anhydride, acyl chloride, ester, amide, aldehyde & ketone)	C=O stretch	strong
2150-2300	Alkyne	C≡C stretch	weak
2175-2350	Nitrile	C≡N stretch	variable
2550-3175	Carboxylic acids	O-H stretch	variable, broad
2700-2850	Aldehyde	C-H stretch	medium
2800-3000	Alkane	C-H stretch	medium
2950-3200	Alkene, aromatics	C-H stretch	medium
3100-3400	Primary and secondary amine, amide	N-H stretch	medium
3175-3300	Alkyne	C-H stretch	narrow, strong
3200-3500	Alcohols, Phenols	O-H stretch	strong, broad

In the table above, = (equal sign) means double bond. ≡ (identical to sign) means triple bond.

Read more about "Analytical Chemistry – Infrared (IR) Spectroscopy [New tab] (https://www.compoundchem.com/2015/02/05/irspectroscopy/)" by Andy Brunning / Compound Interest, CC BY-NC-ND

29.7a Mass spectrometry and a guide to interpreting mass spectra

Mass spectrometry is an analytical technique that allows us to measure the masses of atoms and molecules. The most important peak in a mass spectrum is the molecular ion peak, which can be used to determine the mass of the molecule, but fragment icons can also provide information on chemical structure.

How mass spectrum works:

1. A small sample of the substance to be analyzed is added to the mass spectrometer.
2. The mass spectrometer ionizes the sample. This can be done win a number of ways, including with a laser, applying a voltage to a liquid sample spray, or firing electrons at a gaseous sample. Some molecules fragment into smaller ions.
3. A mass analyzer separates ions based on their mass/charge ratio. This can be done in a number of ways using an electric and/or magnetic field.
4. Ions hit the detector and it converts them into a signal, amplifies it, and records it.
5. The signal is output as a mass spectrum.

Mass Spectrometry – Common Ion Fragments

Ion Fragment	Mass Value	Notes
CH_3^+	15	–
$CH_3CH_2^+$	29	–
$NH_2CH_2^+$	30	–
$HOCH_2^+$	31	–
Cl^+	35/37 (3:1)	Two peaks seen due to the ^{35}Cl and ^{37}Cl isotopes, in a 3:1 ratio due to their natural abundance.
$CH_2{=}CHCH_2^+$	41	–
$CH_3CH_2CH_2^+$	43	–
$CH_3C{=}O^+$	43	–
$NH_2CH_2CH_2^+$	44	–
$NH_2C{=}O^+$	44	–
$ClCH_2^+$	49/51 (3:1)	3:1 ratio due to natural abundance of chlorine.
$CH_3CH_2CH_2CH_2^+$	57	–
$CH_3CH_2C{=}O^+$	57	–
$NH_2CH_2CH_2CH_2^+$	58	–
$CH_3OC{=}O^+$	59	–
$CH_3CH_2OC{=}O^+$	59	–
$CH_3CH_2CH_2CH_2CH_2^+$	71	–
$CH_3CH_2CH_2C{=}O^+$	71	–
$C_6H_5^+$ (phenyl)	77	–
Br^+	79/81 (1:1)	Two peaks seen due to the ^{79}Br and ^{81}Br isotopes, in a 1:1 ratio due to their natural abundance.
$CH_3CH_2CH_2CH_2CH_2CH_2^+$	85	–
$BrCH_2^+$	93/95 (1:1)	1:1 ratio due to natural abundance of bromine.

In the table above, = (equal sign) means double bond.

A selection of common fragment ions seen in mass spectra are shown above, along with their masses. Note

that the structures shown are general representations, and it can also be possible for isomeric structures (those with the same constituent atoms, but a different structure) to cause the peaks in spectra. There are many more fragments possible that those shown, but knowledge of these fragments should suffice to interpret spectra of most simple molecules.

Read more about "Mass spectrometry and a guide to interpreting mass spectra [New tab] (https://www.compoundchem.com/2015/05/07/mass-spectrometry/)" by Andy Brunning / Compound Interest, CC BY-NC-ND

29.9a Analytical Chemistry – A Guide to Proton Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is a commonly used technique for organic compound structure determination. In ^{1}H NMR, applying external magnetic field causes the nuclei spin to flip. The environment of the proton in the molecule affects where the signal is seen on the resultant spectrum.

^{1}H NMR Spectroscopy – Chemical Shift Values

Chemical Shift (δ, ppm)	Functional Group	Structure
0	TMS (reference compound in sample)	n/a
0.5-5.0	Alcohol hydroxyl (0.5-5.0) or amino (1.0-4.0)	R-O-*H or R-N*H_2
0.7-1.3	Primary alkyl	R-C*H_3
1.2-1.5	Secondary alkyl	R-C*H_2-R
1.3-1.8	Tertiary alkyl	R_3-C*H
1.6-2.1	Allylic	R_2-C=CR-C*H_3
2.2-2.7	Ketone	R-C(=O)-C*H_3
2.2-2.7	Benzylic	Ar-C*H_3
2.5-3.2	Acetylenic	R-C≡C-*H
3.2-3.9	Alkyl halide	R-C*H_2-X
3.3-4.0	Alcohol	R-C*H_2-OH
3.4-3.8	Ether	R-C*H_2-O-R
4.5-7.0	Phenolic	Ar-O*H
4.7-5.0	Vinylic	R_2-C=C*H_2
5.5-8.5	Amide	R-C(=O)-NR*H
6.0-8.5	Aromatic	Ar-*H
9.5-10.5	Aldehyde	R-C(=O)*H
10.0-13.0	Carboxylic acid	R-C(=O)-O*H

Key: C means carbon atom. H means hydrogen atom. *H means hydrogens producing signal. O means oxygen atom. N means nitrogen atom. R means rest of organic molecule. Ar means aromatic ring. X means halogen atom. – (minus sign) means single bond. = (equal sign) means double bond. ≡ (identical to sign) means triple bond. Note these are typical values only, and vary depending on the solvent, the temperature, and presence of other functional groups.

Spin-spin coupling patterns in NMR spectra

Hydrogen nuclei themselves possess a small magnetic field, and can influence the signal seen for hydrogen on neighbouring carbon atoms. This is known as spin-spin coupling. The number of signals the original signal is split into is equal to the number of hydrogens on neighbouring carbon atoms plus one, according to

the patterns shown in the table below. The area underneath the peaks indicated the number of hydrogen atoms responsible for each signal.

Spin-spin coupling patterns in NMR spectra

Pattern	Number of hydrogens on adjacent carbon atoms	Number of hydrogen atoms responsible for each signal
Singlet	0 adjacent H	1
Doublet	1 adjacent H	1:1
Triplet	2 adjacent H	1:2:1
Quartet	3 adjacent H	1:3:3:1
Quintet	4 adjacent H	1:4:6:4:1
Sextet	5 adjacent H	1:5:10:10:5:1
Septet	6 adjacent H	1:6:15:20:15:6:1
Octet	7 adjacent H	1:7:21:35:35:21:7:1
Nonet	8 adjacent H	1:8:28:56:70:56:28:8:1

Read more about "Analytical Chemistry – A Guide to Proton Nuclear Magnetic Resonance (NMR) [New tab] (https://www.compoundchem.com/2015/02/24/proton-nmr/)" by Andy Brunning / Compound Interest, CC BY-NC-ND

29.10a A Guide to 13-C Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is a commonly used technique for organic compound structure determination. In ^{13}C NMR, applying an external magnetic field causes the nuclei spin to flip. The environment of the carbon atom in the molecule affects where the signal is seen on the resultant spectrum.

^{13}C NMR Spectroscopy – Chemical Shift Values

Chemical Shift (δ, ppm)	Functional Group	Structure
0	TMS (reference compound in sample)	n/a
0-40	Primary alkyl	R-*CH_3
10-50	Secondary alkyl	R-*CH_2-R
10-65	Alkyl halide	R-*CR_2-X
15-50	Tertiary alkyl	R_3-*C-H
50-65	Alcohol	R_3-*C-OH
50-75	Ether or ester	R_3-*C-O-R
65-90	Alkyne	R-C≡*C-H
65-90	Nitro compound	R_3-*C-NO_2
100-120	Alkene	R_2C=*CH_2
100-155	Aromatic carbons	R_5C_5*C-H
115-135	Nitrile	R-*C≡N
120-140	Alkene	R_2*C=CH_2
160-180	Acyl chloride	R-*C(=O)-X
160-180	Amide	R-*C(=O)-NR_2
160-180	Ester	R-*C(=O)-OR
170-180	Carboxylic acid	R-*C(=O)-OH
185-210	Aldehyde	R-*C(=O)-H
200-220	Ketone	R-*C(=O)-R

Key: C means carbon atom. *C means carbon causing signal. H means hydrogen atom. O means oxygen atom. N means nitrogen atom. R means rest of organic molecule. Ar means aromatic ring. X means halogen atom. – (minus sign) means single bond. = (equal sign) means double bond. ≡ (identical to sign) means triple bond. Note these are typical values only, and vary depending on the solvent, the temperature, and presence of other functional groups.

^{12}C 99%, ^{13}C 1%

Only 1% of carbon atoms are carbon-13, atoms which have one more neutron that carbon-12. NMR doesn't work for carbon-12, as its nucleus doesn't have a 'spin'. The frequency required to 'flip' a carbon-13 nucleus is around a quarter of that required to flip a hydrogen nucleus in H-NMR. As the probability of two

adjacent carbons in a single molecule being carbon-13 atoms is very low, no splitting of peaks is seen, unlike in H-NMR.

Read more about "A Guide to 13-C Nuclear Magnetic Resonance (NMR) [New tab] (https://www.compoundchem.com/2015/04/07/carbon-13-nmr/)" by Andy Brunning / Compound Interest, CC BY-NC-ND

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APPENDIX A: KEY ELEMENT INFORMATION

Key Element Information

Organic chemistry is based on the element carbon and several other key elements. It is helpful to remember some key information about these elements while learning about organic compounds. Table A highlights some key information as well as links to more information from our *Enhanced Introductory College Chemistry* (https://ecampusontario.pressbooks.pub/enhancedchemistry/) book.

Table A: Key information about carbon, hydrogen, oxygen, nitrogen and halogen elements.

Element	Carbon	Hydrogen	Oxygen	Nitrogen	Halogen	More Info
Symbol	C	H	O	N	F, Cl, Br, I	3.1 Elements (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/elements/)
Atomic Number	6	1	8	7	9, 17, 35, 53	Appendix A: The Periodic Table (https://ecampusontario.pressbooks.pub/enhancedchemistry/back-matter/periodic-table/)
Atomic Mass	12	1	16	14	19, 35, 80, 127	5.6 Atomic Mass (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/atomic-mass/)
Number of Protons	6	1	8	7	9, 17, 35, 53	5.4 Defining the Nuclear Atom (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/nuclear-atom/)
Number of Electrons	6	1	8	7	9, 17, 35, 53	5.4 Defining the Nuclear Atom (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/nuclear-atom/)
Number of Neutrons	6	0	8	7	10, 18, 45, 74	5.4 Defining the Nuclear Atom (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/nuclear-atom/)
Natural Isotopes	^{12}C, ^{13}C, ^{14}C	^{1}H, ^{2}H, ^{3}H	^{16}O, ^{17}O, ^{18}O	^{14}N, ^{15}N	see more info	5.5 Isotopes of the Elements (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/isotopes-elements/)
Number of Valence Electrons	4	1	6	5	7 (all)	10.5 Atomic Structures of the First 20 Elements (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/structures-first20-elements/)
Electron Configuration	$1s^2 2s^2 2p^2$	$1s^1$	$1s^2 2s^2 2p^4$	$1s^2 2s^2 2p^3$	$1s^2 2s^2 2p^5$ (F), ...$s^2 p^5$ (Cl, Br, I)	10.5 Atomic Structures of the First 20 Elements (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/structures-first20-elements/)
Lewis Structure						11.3 Lewis Symbols and Structures (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/lewis-symbols-structures/)

Typical Ion	no ion formed (covalent bonding)	H^+ or H^-	O^{2-}	N^{3-}	F^-, Cl^-, Br^-, I^-	5.4 Defining the Nuclear Atom (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/nuclear-atom/)
Electronegativity	2.5	2.1	3.5	3.0	4.0, 3.0, 2.8, 2.5	11.2 Covalent Bonding (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/covalent-bonding/)
Typical Number of Bonds	4	1	2	3	1	11.6 Molecular Structure and Polarity (https://ecampusontario.pressbooks.pub/enhancedchemistry/chapter/molecular-structure-polarity/)

Attribution & References

Images are used from:

- The Lewis structure of a carbon atom by K!roman, SVG: Marlus_Gancher, PDM
- Lewis structure of the hydrogen atom by Ricordisamoa, PDM
- Lewis dot diagram for oxygen by Adrignola, CC0
- Lewis structure for nitrogen – 窒素の価電子.svg by すじにくシチュー, CC0
- Lewis structure for chlorine by Apostoloff, , PDM

APPENDIX B: ORGANIC CHEMISTRY SOFTWARE

Learning organic chemistry is like learning a new language. Part of the learning is learning how to draw the structure of organic molecules. There are software tools to help digitize drawings.

> Any software mentioned in this book is available via the web and typically free of charge. The authors have no financial nor ownership stake in the software. Any changes in availability or usability of the software are outside the scope of the authors' influence.

Organic Chemistry Molecule Drawing Software

There are some suggested molecule drawing tools.

MolView

To access Molview, visit MolView (https://molview.org/). This is a web-based system. Figure B1 shows examples of the potential output from MolView.

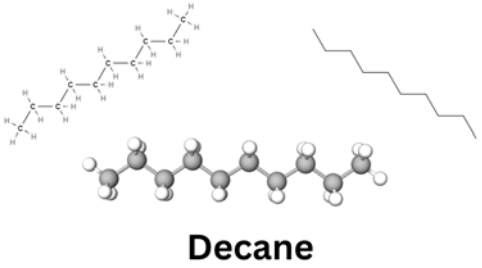

Figure B1: Structural diagram, line structure and 3D model for decane ($C_{10}H_{22}$). Drawn with MolView.org

For a step-by-step tutorial, **watch Chemistry Unit 4: Using MolView to Construct 3D Models of Organic Molecules – YouTube (https://youtu.be/TR17qnrRRqA?) (7 min).**

BIOVIA Draw

To download BIOVIA Draw, visit BIOVIA Draw for Academics – Dassault Systèmes (3ds.com) (https://discover.3ds.com/biovia-draw-academic). BIOVIA Draw has been known under a variety of names including Accelyrs Draw. It is a computer-based system that is downloaded. It can be integrated into computer-based Microsoft products. Figure B2 shows examples of the potential output from BIOVIA Draw.

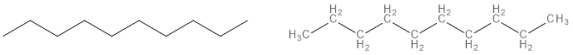

Figure B2: Line structure and condensed structural diagram for decane ($C_{10}H_{22}$). Drawn with BIOVIA Draw.

For a step-by-step tutorial, **watch Biovia draw tutorial – YouTube (https://youtu.be/kf0tdZxsr3A?) (7 min).**

ChemSketch

To download ChemSketch, visit Free Chemical Drawing Software for Students | ChemSketch | ACD/Labs (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/). It is a computer-based system that is downloaded.

For a step-by-step tutorial, **watch How to Use ChemSketch from ACD/Labs (Tutorial and Demo) – YouTube (https://youtu.be/l06ljePcg8U?) (7 min).**

Organic Chemistry Lab Drawing Software

Looking to draw a lab setup or visual step-by-step instructions with chemistry lab equipment, try: Chemix – Draw Lab Diagrams. Simply. (https://chemix.org/) See Figure B3 for an example of the types of chemical equipment and style of diagrams that can be made.

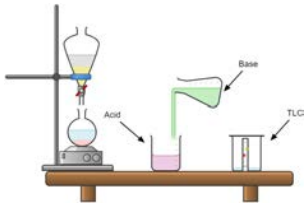

Figure B3: Example of Chemix.org lab setup diagram (Drawn using www.chemix.org)

Attribution & References