NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: CHM 309

COURSE TITLE: ORGANIC SPECTROSCOPY

COURSE GUIDE

CHM 309

Organic SPECTROSCOPY

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INTRODUCTION

CHM 309: APPLIED SPECTROSCOPY is a 2 credit course for BSc Chemistry.

The course is broken down into 3 modules of 13 study units. At the end of this course, a student is expected to be conversant with the principles of Ultraviolet/Visible (UV), Infra-red (IR), Nuclear magnetic resonance (NMR) and Mass spectroscopic methods. The student should also be familiar with the instruments used in these methods and the interpretation of data generated from these techniques.

WHAT YOU WILL LEARN IN THIS COURSE

You will learn about the principles of UV/Vis, Infra-red, NMR and Mass spectroscopic methods. You will also learn the operations of the instruments involved in these analytical techniques and their application in structure elucidation.

COURSE AIMS

The aim of this course is for you to understand the principles and operation of the instruments involved in UV/Vis, Infra-red, NMR and MS spectroscopic methods. You will also learn to apply the principles in the analysis of organic molecules.

COURSE OBJECTIVES

In order to achieve the course aims, there are some overall objectives set for the course. Besides, each module and each unit has their respective objectives which you and your facilitator must constantly refer to, so that no objective is skipped. All the modules and unit objectives are specifics of the course objectives. The course objectives are stated as follows:

- To understand the principles of UV/Vis, Infra-red, NMR and MS spectroscopic methods.
- To become familiar with the instruments involved in UV/Vis, Infra-red, MS and NMR spectroscopic methods.
- To be able to apply the general rules learnt to interpret data generated from the instruments.

- To be able to apply these analytical methods in solving various analytical problems.

WORKING THROUGH THIS COURSE

This course contains some packages that you will be given at the beginning of the semester: one of them is the course material. Your full participation in both the continuous assessment and the final written examination are two areas expected of you to fulfil at the end of the course. Stated below are the components of this course and what you have to do.

COURSE MATERIAL

Major course materials for the course are as follows:

i. **Course guide**: This looks like a blue print that spells out what constitutes the course itself.

ii **Study units**: Each of these provides an overview of the content and number of units that will be covered in this course.

iii) **Assignment files:** These files contain challenging tutorial questions termed Tutor-Marked Assignment (TMAs) that will enable you to assess yourself at the end of every assignment that will be handed out by your tutor.

iv) **Presentation schedule**: Certainly, the modus operandis (e.g. time table, hours expected on each Unit/ Module, assignment submission procedure on how it will be self tutored with the monitoring techniques by NOUN will be in the information package of this schedule).

STUDY UNITS

There are 13 study units and 3 modules in this course. They are:

MODULE 1 ULTRAVIOLET/VISIBLE AND INFRARED SPECTROSCOPY

Unit 1: Principles of Ultraviolet/Visible Spectroscopy

Unit 2: Applications of UV/Visible Spectroscopy

Unit 3: Principles of Infrared Spectroscopy

Unit 4: Applications of Infrared Spectroscopy

MODULE 2 MASS SPECTROMETRY

Unit 1: Principles of Mass spectrometry

Unit 2: Sample introduction, Ionization techniques and mass analyzers used in mass spectrometry

Unit 3: The mass spectrum and Interpretation of a mass spectrum

Unit 4 Applications of Mass Spectrometry

MODULE 3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Unit 1 Principles of Nuclear Magnetic Spectroscopy

Unit 2 Proton (^{1}H)- NMR

Unit 3 Carbon (^{13}C)- NMR

Unit 4 Two dimensional NMR and other applications of NMR

Unit 5 Structure elucidation of organic molecules with worked examples.

From all indications, you should be able to complete two credit units about 15 weeks in a semester. Well spread out in each unit is: Introduction to the unit, specific objectives, body of the unit, conclusion, summary, Tutor Marked Assignments and References.

Details of the study units have earlier been presented. It is spelt out in modules with corresponding units and titles. You will be expected to spend 2-3 hours in studying a unit.

Recommended Texts

These texts will be of immense benefit to this course:

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 189-270.
2. William Kemp (1991) Organic spectroscopy, W.H. Freemans & Co.
3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Ltd, Essex, England (1st ed).* 159-184.

Assignment File

The assignment file will be given to you in due course. In this file, you will find all the details of the work you must submit to your tutor for marking. The marks you obtain for these assignments will count towards the final mark for the course. Altogether, there are 15 tutor marked assignments for this course.

PRESENTATION SCHEDULE

The presentation schedule included in this course guide provides you with important dates for completion of each tutor marked assignment. You should therefore try to meet the deadlines.

ASSESSMENT

There are two aspects to the assessment of this course. First, there are tutor marked assignments and second, the written examination.

You will be expected to complete at least ten assignments by the end of the course. Some of these will be in the form of a project and continuous assessment (CA). You will be expected to write a final examination in the course. The overall score in the course will be a sum of 40% of CA and 60% of written examination. You will be expected to have 50% in the CA and 50% in the written examination; anything short of this will count as failure.

TUTOR-MARKED ASSIGNMENT

There are 13 TMAs in this course. You need to submit all the TMAs. The best 4 will therefore be counted. When you have completed each assignment, send them to your tutor as soon as possible and make sure that it gets to your tutor on or before the stated deadline. If for any reason you cannot complete your assignment on time, contact your tutor before the assignment is due to discuss the possibility of extension. Extension will not be granted after the deadline, unless on exceptional cases.

FINAL EXAMINATION AND GRADING

The end of course examination for Applied spectroscopy will be for about 3 hours and it has a value of 60% of the total course work. The examination will consist of questions, which will reflect the type of self-testing, practice exercise and tutor-marked assignment problems you have previously encountered. All areas of the course will be assessed.

Use the time between finishing the last unit and sitting for the examination to revise the whole course. You might find it useful to review your self-test, TMAs and comments on them before the examination. The end of course examination covers information from all parts of the course.

COURSE MARKING SCHEME

Assignment	Marks
Assignments 1-13	13 assignments, 40% for the best 4 Total = 10% x 4 = 40%
End of course examination	60% of overall course marks
Total	100% of course materials

COURSE OVERVIEW

This table indicates the units, the number of weeks required to complete them and the assignments.

Unit	Title of Work	Weeks Activity	Assessment (End of Unit)
	Course Guide	Week 1	
Module 1	**Ultraviolet/Visible (UV) Spectroscopy and Infrared (IR) Spectroscopy**		
Unit 1	Principles of UV/Visible Spectroscopy	Week 1	Assignment 1
Unit 2	Applications of UV/Vis Spectroscopy	Week 2	Assignment 2
Unit 3	Principles of Infrared Spectroscopy	Week 3	Assignment 3
Unit 4	Applications of Infrared Spectroscopy	Week 4	Assignment 4
Module 2	**Mass Spectrometry**		
Unit 1	Principles of Mass spectrometry	Week 5	Assignment 5
Unit 2	Sample Introduction, Ionization technique and Interpretation of the mass spectrum	Week 6	Assignment 6
Unit 3	Interpretation of the Mass spectrum	Week 7	Assignment 7
Unit 4	Applications of Mass spectrometry	Week 8	Assignment 8
Module 3	**Nuclear Magnetic Resonance (NMR) Spectroscopy**		
Unit 1	Principles of Nuclear Magnetic Spectroscopy	Week 9	Assignment 9
Unit 2	Proton NMR	Week 10	Assignment 10
Unit 3	Carbon NMR	Week 11	Assignment 11
Unit 4	Two dimensional NMR and other applications of NMR Spectroscopy	Week 12	Assignment 12
Unit 5	Structure elucidation of organic molecules with worked examples.	Week 13	Assignment 13

HOW TO GET THE MOST OUR OF THIS COURSE

In distance learning, the study units replace the university lecturer. This is one of the huge advantages of distance learning mode; you can read and work through specially designed study materials at your own pace and at a time and place that suit you best. Think of it as reading form the teacher, the study guide tells you what to read, when to read and the relevant texts to consult. You are provided exercises at appropriate points, just as a lecturer might give you an in-class exercise.

Each of the study units follows a common format. The first item is an introduction to the subject matter of the unit and how a particular unit is integrated with the other units and the course at a whole. Next to this is a set of learning objectives. These learning objectives are meant to guide your studies. The moment a unit is finished, you must go back and check whether you have achieved the objectives. If this is made a habit, then you will significantly improve your chances of passing the course. The main body of the units also guides you through the required readings from other sources. This will usually be either from a set of books or from other sources.

Self assessment exercises are provided throughout the unit, to aid personal studies and answers are provide at the end of the unit. Working through these self tests will help you to achieve the objectives of the unit and also prepare you for tutor marked assignments and examinations. You should attempt each self test as you encounter them in the units.

The following are practical strategies for working through this course

1. Read the course guide thoroughly.
2. Organize a study schedule. Refer to the course overview for more details. Note the time you are expected to spend on each unit and how the assignment relates to the units. Important details, e.g. details of your tutorials and the date of the first day of the semester are available. You need to gather together all these information in one place such as a diary, a wall chart calendar or an organizer. Whatever method you choose, you should decide on and write in your own dates for working on each unit.
3. Once you have created your own study schedule, do everything you can to stick to it. The major reason that students fail is that they get behind with their course works. If

you get into difficulties with your schedule, please let your tutor know before it is too late for help.

4. Turn to Unit 1 and read the introduction and the objectives for the unit.
5. Assemble the study materials. Information about what you need for a unit is given in the table of content at the beginning of each unit. You will almost always need both the study unit your are working on and one of the materials recommended for further readings, on your desk at the same time.
6. Work through the unit, the content of the unit itself has been arranged to provide a sequence for you to follow. As you work through the unit, you will be encouraged to read from your set books.
7. Keep in mind that you will learn a lot by doing all your assignments carefully. They have been designed to help you meet the objectives of the course and will help you pass the examination.
8. Review the objectives of each study unit to confirm that you have achieved them. If you are not certain about any of the objectives, review the study material and consult your tutor.
9. When you are confident that you have achieved a unit's objectives, you can start on the next unit. Proceed unit by unit through the course and try to pace your study so that you can keep yourself on schedule.
10. When you have submitted an assignment to your tutor for marking, do not wait for its return before starting on the next unit. Keep to your schedule. When the assignment is returned, pay particular attention to your tutor's comments, both on the tutor marked assignment form and also written on the assignment. Consult your tutor as soon as possible if you have any questions or problems.
11. After completing the last unit, review the course and prepare yourself for the final examination. Check that you have achieved the unit objectives (listed at the beginning of each unit) and the course objectives (listed in this course guide).

FACILITATORS/TUTORS AND TUTORIALS

There are 8 hours of tutorials provided in support of this course. You will be notified of the dates, times and location of these tutorials as well as the name and phone number of your facilitator, as soon as you are allocated a tutorial group.

Your facilitator will mark and comment on your assignments, keep a close watch on your progress and any difficulties you might face and provide assistance to you during the course. You are expected to mail your Tutor Marked Assignment to your facilitator before the schedule date (at least two working days are required). They will be marked by your tutor and returned to you as soon as possible.

Do not hesitate to contact your facilitator by telephone or e-mail if you need assistance. The following might be circumstances I which you would find assistance necessary, hence you would have to contact your facilitator if:

1. You do not understand any part of the study or the assigned readings.
2. You have difficulty with the self-tests
3. You have a question or problem with an assignment, with your tutor's comments or with the grading of an assignment.

You should endeavour to attend the tutorials. This is the only chance to have face to face contact with your course facilitator and to ask questions which are answered instantly. You can raise any problem encountered in the course of your study.

To gain much benefit from course tutorials, prepare a question list before attending them. You will learn a lot from participating actively in discussions. GOODLUCK!

Course Code CHM 309

Course Title: Organic Spectroscopy

Course Developer: Dr Gloria Abiodun Ayoola

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Course Editor:

Programme Leader:

Course Co-ordinator

NATIONAL OPEN UNIVERSITY OF NIGERIA

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INTRODUCTION

Spectroscopy is widely used in structure determination and in the qualitative and quantitative analysis of substances. All forms of spectroscopy involve the interaction of a sample with a form of energy. It is the way in which this energy is assimilated that gives information concerning the identity and quantity of the chemical species present in the sample. This course discusses only the most widely used forms of spectroscopy, namely, ultraviolet/ visible, infra-red and nuclear magnetic resonance spectroscopy. We will also discus mass spectrometry which is not a form of spectroscopy but is usually treated as such.

WHAT YOU WILL LEARN IN THIS COURSE

You will learn about the principles of UV/Vis spectroscopy, Infra-red, NMR and Mass spectroscopic methods. You will also learn the operations of the instruments involved in these analytical techniques and their application in structure elucidation.

COURSE AIMS

The aim of this course is for you to understand the principles and operation of the instruments involved in UV/Vis, Infra-red, NMR and MS spectroscopic methods. You will also learn to apply the principles in the analysis of organic molecules.

COURSE OBJECTIVES

In order to achieve the course aims, there are some overall objectives set for the course. Besides, each module and each unit has their respective objectives which you and your facilitator must constantly refer to, so that no objective is skipped. All the modules and unit objectives are specifics of the course objectives. The course objectives are stated as follows:

i) To understand the principles of UV/Vis, Infra-red, NMR and MS spectroscopic methods.

ii) To become familiar with the instruments involved in UV/Vis, Infra-red, MS and NMR spectroscopic methods.

iii) To be able to apply the general rules learnt to interpret data generated from the instruments.

iv) To be able to apply these analytical methods in solving various analytical problems.

WORKING THROUGH THIS COURSE

This course contains some packages that you will be given at the beginning of the semester: one of them is the course material. Your full participation in both the continuous assessment and the final written examination are two areas expected of you to fulfil at the end of the course. The 13 units of the course packaged for you in modules are shown below;

MODULE 1 ULTRAVIOLET/VISIBLE AND INFRARED SPECTROSCOPY

Unit 1: Principles of Ultraviolet/Visible Spectroscopy

Unit 2: Applications of UV/Visible Spectroscopy

Unit 3: Principles of Infrared Spectroscopy

Unit 4: Applications of Infrared Spectroscopy

MODULE 2 MASS SPECTROMETRY

Unit 1: Principles of Mass spectrometry

Unit 2: Sample Introduction, Ionization technique and mass analyzers used in mass spectrometry

Unit 3: The mass spectrum and Interpretation of a mass spectrum

Unit 4 Applications of Mass Spectrometry

MODULE 3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Unit 1 Principles of Nuclear Magnetic Spectroscopy

Unit 2 Proton (^{1}H)- NMR

Unit 3 Carbon (^{13}C)- NMR

Unit 4 Two dimensional NMR and other applications of NMR spectroscopy

Unit 5 Structure elucidation of organic molecules with worked example

From all indications, you should be able to complete two credit units in about 15 weeks in a semester. Well spread out in each unit are: Introduction to the unit, specific objectives, body of the unit, conclusion, summary, Tutor Marked Assignments and References.

COURSE MATERIAL

Major course materials for the course are as follows:

i. **Course guide**: This looks like a blue print that spells out what constitutes the course itself.

ii **Study units**: Each of these provides an overview of the content and number of units that will be covered in this course.

iii) **Assignment files:** These files contain challenging tutorial questions termed Tutor-Marked Assignment (TMAs) that will enable you to assess yourself at the end of every assignment that will be handed out by your tutor.

iv) **Presentation schedule**: Certainly, the modus operandis (e.g. time table, hours expected on each unit/ Module, assignment submission procedure on how it will be self tutored with the monitoring techniques by NOUN will be in the information package of this schedule).

STUDY UNITS

Details of the study units have earlier been presented. It is spelt out in modules with corresponding units and titles. You will be expected to spend 2-3 hours in studying a unit.

REFERENCES AND OTHER RESOURCE

Apart from this study unit, some reference materials are provided as additional reading materials to support your study. You may come across them in NOUN library or elsewhere.

INSTRUCTIONAL MEDIA

As an open and distance learning University, several and relevant multi-media that can make learning possible are available.

ASSIGNMENT FILE

This has been discussed earlier. It is mandatory to always turn in your assignments to any tutor assigned.

ASSESSMENT

You will be expected to complete at least ten assignments by the end of the course. Some of these will be in the form of a project and continuous assessment (CA). You will be expected

to write a final examination in the course. The overall score in the course will be a sum of 40% of CA and 60% of written examination. You will be expected to have 50% in the CA and 50% in the written examination; anything short of this will count as failure.

TUTOR-MARKED ASSIGNMENT

There are 13 TMAs in this course. You need to submit all the TMAs. The best 4 will therefore be counted. When you have completed each assignment, send them to your tutor as soon as possible and make sure that it gets to your tutor on or before the stated deadline. If for any reason you cannot complete your assignment on time, contact your tutor before the assignment is due to discuss the possibility of extension. Extension will not be granted after the deadline, unless on exceptional cases

FINAL EXAMINATION AND GRADING

The end of course examination for introduction to CHM 309 will be for about 3 hours and it has a value of 60% of the total course work. The examination will consist of questions, which will reflect the type of self-testing, practice exercise and tutor-marked assignment problems you have previously encountered. All areas of the course will be assessed.

Use the time between finishing the last unit and sitting for the examination to revise for the whole course. You might find it useful to review your self-test, TMAs and comments on them before the examination. The end of course examination covers information from all parts of the course.

COURSE MARKING SCHEME

Assignment	**Marks**
Assignment 1-15	13 assignments, 40% for the best 4 Total = 10% x 4 = 40%
End of course examination	60% of overall course marks
Total	100% of course materials

FACILITATORS/TUTORS AND TUTORIALS

There are 16 hours of tutorials provided in support of this course. You will be notified of the dates, times and location of these tutorials as well as the name and phone number of your facilitator, as soon as you are allocated a tutorial group.

Your facilitator will mark and comment on your assignments, keep a close watch on your progress and any difficulties you might face and provide assistance to you during the course. You are expected to mail your Tutor Marked Assignment to your facilitator before the schedule date (at least two working days are required). They will be marked by your tutor and returned to you as soon as possible.

Do not delay to contact your facilitator by telephone or e-mail if you need assistance. The following might be circumstances in which you would find assistance necessary, hence you would have to contact your facilitator if:

4. You do not understand any part of the study or the assigned readings.
5. You have difficulty with the self-tests
6. You have a question or problem with an assignment or with the grading of an assignment.

You should endeavour to attend the tutorials. This is the only chance to have face to face contact with your course facilitator and to ask questions which are answered instantly. You can raise any problem encountered in the course of your study.

To gain much benefit from course tutorials, prepare a question list before attending them. You will learn a lot from participating actively in discussions.

SUMMARY

Spectroscopic methods are invaluable in solving analytical chemistry problems. They have a broad application in chemical, pharmaceutical and various other industries and in research. The methods discussed here complement each other in solving analytical problems. Upon completion of this course you will be equipped in how to use data from each of the analytical techniques either separately or in combination to obtain useful information regarding the molecules being analyzed. You will be able to answer the following questions:

1. What is UV/Vis spectroscopy?
2. What is the UV/Vis region of the electromagnetic spectrum?
3. Which compounds absorb light in the UV/Vis region?
4. What is a chromophore?
5. What is a bathochromic or hyperchromic shift
6. How can I use UV absorbance in quantitative and qualitative analysis?

7. What is infra-red spectroscopy?
8. At which IR region do various functional groups absorb?
9. Which instruments are used in IR spectroscopy?
10. How can I interpret an IR spectrum?
11. What is proton NMR?
12. What is carbon NMR?
13. What is the meaning of ‘chemical shift’?
14. How can I interpret an NMR spectrum?
15. What is mass spectrometry?
16. Which instruments are used in MS?
17. What is a base peak?
18. What is a molecular ion?
19. How can I interpret a mass spectrum

Of course, the list of questions that you can answer is not limited to the above list.

I wish you success in the course and I hope that you will find it both interesting and useful.

CHM 309

MODULE 1 ULTRAVIOLET/VISIBLE AND INFRARED SPECTROSCOPY

Unit 1: Principles of Ultraviolet/Visible Spectroscopy

Unit 2: Applications of UV/Visible Spectroscopy

Unit 3: Principles of Infrared Spectroscopy

Unit 4: Applications of Infrared Spectroscopy

MODULE 2 MASS SPECTROMETRY

Unit 1: Principles of Mass spectrometry

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MODULE 3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Unit 1 Principles of Nuclear Magnetic Spectroscopy

Unit 2 Proton (^{1}H)- NMR

Unit 3 Carbon (^{13}C)- NMR

Unit 4 Two dimensional NMR and other applications of NMR

Unit 5 Structure elucidation of organic molecules with worked example

MODULE 1: ULTRAVIOLET/VISIBLE AND INFRARED SPECTROSCOPY

Unit 1: Principles of UV/Visible Spectroscopy

1.0 Introduction

The interaction between radiations and matter is a fascination one. Most drug molecules absorb radiation in the ultraviolet region of the spectrum, although some are coloured and thus absorb radiation in the visible region. Absorption in the Ultraviolet and visible regions of the electromagnetic spectrum corresponds to transitions between electronic energy levels and provides useful analytical information for both organic and inorganic samples.

2.0 Objectives

At the end of this unit, you will:

- Understand the principles of UV/Vis absorption spectroscopy
- Know about the different components of a UV spectrophotometer
- Know the factors that govern absorption of radiation in the UV/Vis region
- Know how to apply the measurement of the relationship between concentration and absorption in quantitative analysis using the Beer-Lambert Law.

3.0 Main content

3.1 Absorption Spectroscopy

The absorption of UV/Vis radiation occurs through excitation of electrons within the molecular structure to a higher energy state. Radiation is a form of energy and we are constantly reminded of its presence via our sense of sight and ability to feel radiant heat. Radiation can be considered either as a continuous wave travelling through space, or as discrete photons of the same energy. The wave approach is more useful for many spectrometric approach. It may be considered in terms of a wave motion where the wavelength, λ, is the distance between two successive peaks (Figure 1.1). The frequency, v, is the number of peaks passing a given point per second. These terms are related in the equation below:

$$c = v\lambda \qquad (1.1)$$

where c is the velocity of light in a vacuum.

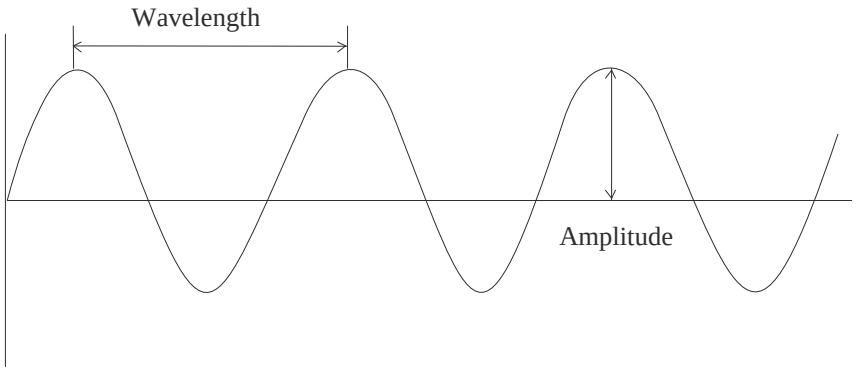


Figure 1.1 The wavelength and amplitude of a wave. Reproduced from James W. Robinson et al., Undergraduate Instrumental Anaylsis CRC Press, Taylor & Francis Group, New Yourk. p65-80.

The standard unit of wavelength is expressed in nanometres. Other units which may be encountered, but whose use is now discouraged are Angstrom and the mill micron (mμ). In some cases, it is more convenient to consider light as a stream of particles called photons. Photons are characterised by their energy, E. The energy of a photon is related to the frequency of light by the equation below:

$$E = hv \qquad (1.2)$$

Where E is the energy in joules (J), h is Planck's constant, 6.626 x 10-34 Js. And v is the frequency in inverse seconds (Hz). From equations (1.1) and (1.2) we can deduce that:

$$E = hc/\lambda \qquad (1.3)$$

From the equations above, we can see that the energy of electromagnetic radiation is directly proportional to its frequency and inversely proportional to its wavelength. Electromagnetic radiation ranges from very low energy (long wavelength, low frequency) radiation, like radio waves and microwaves, to very high energy (short wave-spectrum of interest to us as analytical chemists are shown in Figure 1.2 below. It is clear from this figure that the electromagnetic spectrum to which the human eye responds, is only a very small portion of all radiant energy.

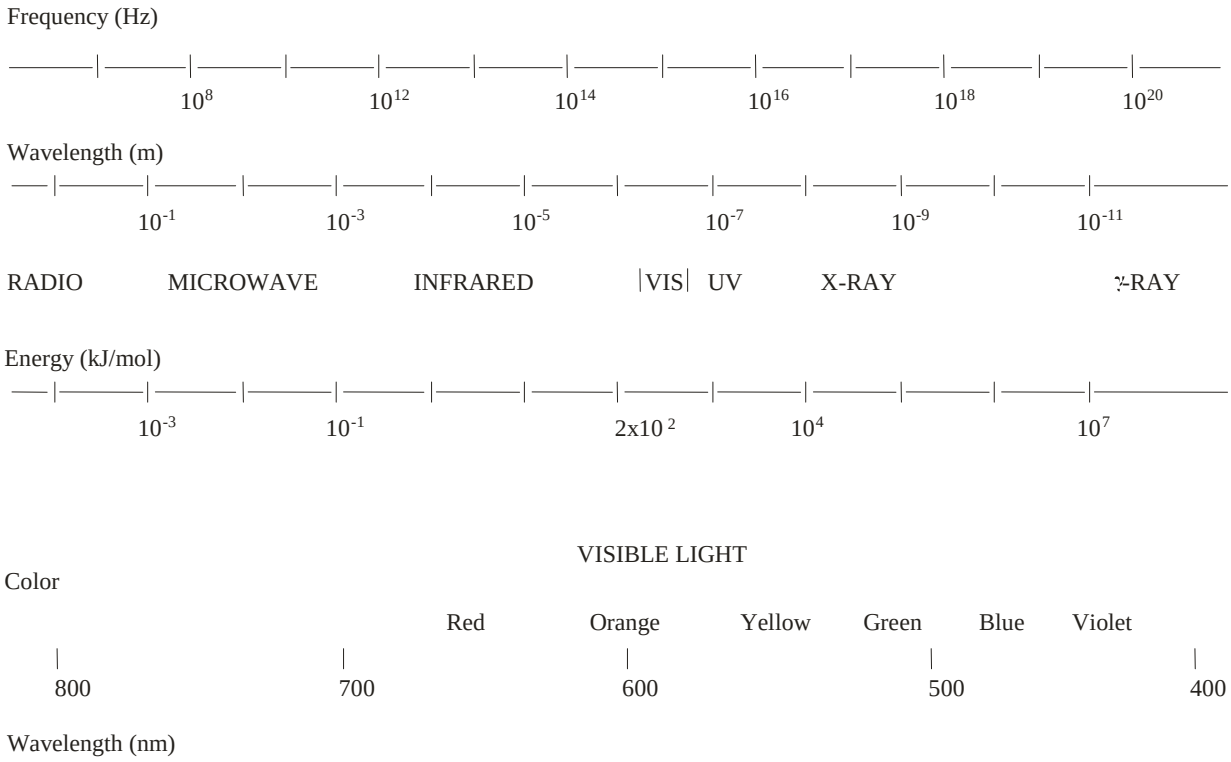


Figure 1.2 Electromagnetic spectrum. Reproduced from James W. Robinson et al., Undergraduate Instrumental Anaylsis CRC Press, Taylor & Francis Group, New York.

Spectroscopy is the study of the interaction of light with matter. Matter is defined as materials composed of molecules or atoms or ions. When light strikes a sample of matter, the light may be absorbed by the sample, transmitted through the sample, reflected off the surface of the sample, or scattered by the sample. Samples can also emit light after absorbing incident light; such a process is called luminescence.

The Ultraviolet (UV) and visible region of the electromagnetic radiation covers the wavelength range from about 100 nm to about 800 nm. The vacuum ultraviolet region, which has the shortest wavelengths and highest energies (100-200 nm) is difficult to make measurements in and is relatively uninformative. Useful ultra-violet and visible absorption spectra are produced by the absorption of electromagnetic radiation with wavelengths in the 200-400 nm (UV) and 400 to 800 nm (Visible) regions of the electromagnetic radiation.

An atom consists of a nucleus surrounded by electrons. Every element has a unique number of electrons, equal to its atomic number for a neutral atom of that element. The electrons are located in atomic orbital's of various types and energies and the electronic energy states of atoms are quantized. The lowest energy, most stable electron configuration of an element is its ground state. The ground state is the normal electron configuration predicted from the 'rules' for filling a many-electron atom. These rules are based on the location of the atom in the periodic table, the aufbau principle, the Pauli exclusion principle and Hund's rule. For example, the ground state electronic configuration for sodium, atomic no 11, is $1s^2 2s^2 2p^6 3s^1$

based on its position in the third row, first group of the periodic table and the requirement to account for 11 electrons. If energy of the right magnitude is provided to an atom, the energy may be absorbed and an outer (valence) electron promoted from the ground state orbital it is in, to a higher energy orbital. The atom is now in a higher energy, less stable, excited state. The electron will return spontaneously to the ground state, because the excited state is less stable than the ground state. In the process, the atom will emit energy; the energy will be equivalent in magnitude to the difference in energy levels between the ground and excited states.

The energy states associated with molecules, like those of atoms are also quantized. When atoms combine to form molecules, the individual atomic orbital's combine to form a new set of molecular orbital's. Molecular orbital's with electron density in the plane of the bonded nuclei, that is, along the axis connecting the bonded nuclei, are called sigma (σ) orbital's. Those molecular orbital's with electron density above and below the plane of the bonded nuclei are called pi (π) orbital's. Sigma and pi orbital's may be of two types, bonding or antibonding orbital's. As an example, the atomic orbital's of carbon, hydrogen and oxygen combine in the molecule of propanone, C_3H_6O (Fig 1.3), so the three carbons are linked in a chain by single (σ) bonds, the two outer carbons are each linked by σ bonds to three hydrogens, while the central carbon is linked by a double bond to the oxygen, that is by both a σ and π bond. Additionally, the oxygen still has unpaired or nonbonded n electrons. This results in a set of bonding and corresponding antibonding electronic orbital's or energy levels. Bonding energies are lower in energy than the corresponding antibonding orbital's. Transitions may occur selectively between these levels, for example between π and π* levels.

Under normal conditions of temperature and pressure, the electrons in the molecule are in the ground state configuration, filling the lowest energy molecular orbital's available. Absorption of the appropriate radiant energy may cause an outer electron to be promoted to a higher energy excited state. As was the case with atoms, the radiant energy required to cause electronic transitions in molecules lies in the visible and UV regions. The excited state of a molecule is less stable than the ground state as with atoms, the molecules will spontaneously revert (relax) to the ground state emitting UV or visible radiant energy. Unlike atoms, the energy states in molecules have rotational and vibrational sublevels, so when a molecule is excited electronically, there is often a simultaneous change in the vibrational and rotational energies. The total energy change is the sum of the electronic, rotational and vibrational energy changes. However, in the condensed states of solid and liquid, rotation is restricted.

Organic molecules contain carbon-carbon bonds, and bonds between carbon and other elements such as hydrogen, oxygen, nitrogen, sulphur, phosphorus and the halogens. Single bonds correspond to the bonding σ orbital, which has an associated antibonding σ* orbital. Multiple bonds may also be formed and corresponds to the π bonding and π* antibonding orbitals. Bonding orbitals have lower energy, while antibonding orbitals have higher energy.

Lone pair of electrons on atoms such as oxygen are little changed in energy. Thus, a molecule such as propanone (acetone) has the structure below (Figure 1.3).

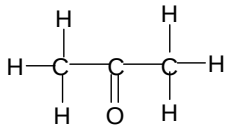


Figure 1.3 Structure of propanone

Figure 1.4 shows that the σ-σ* transitions require the largest energy change and occur at the lowest wavelengths, usually less than 190 nm, which is below the wavelengths measureable with most laboratory instrumentation. The π-π* transitions are very important, as they occur in all molecules with multiple bonds and with conjugated structures, such as aromatic compounds. The transitions occur around 200 nm, but the greater the extent of the conjugation, the closer the energy levels and the higher the observed absorption wavelength. Transitions involving the lone pairs on heteroatoms such as oxygen or nitrogen may be n-σ*, which occur around 200nm, or n-π*, which occur near 300 nm. These values are considerably altered by the specific structure and the presence of substituent (auxochromes) in the molecules.

The single C-H and C-C bond relate to σ orbitals, the carbonyl double bond to the π orbitals and the unpaired electrons on the oxygen to the nonbonding n-levels. The energy levels may be grouped approximately as shown in Fig 1.4. Transitions between σ and σ* levels and between π and π* are favoured and those of the n electrons to the higher levels also occur

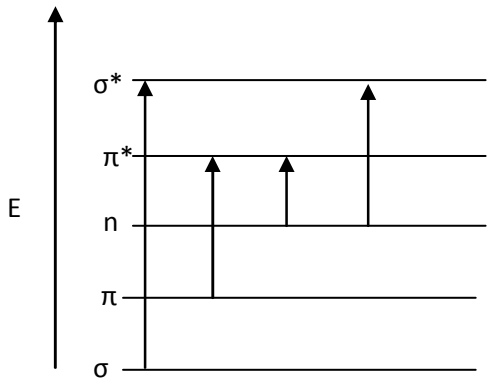


Fig. 1.4. Typical transitions for organic molecules.

The energy levels involved in transitions in the UV-visible region are the electronic levels of atoms and molecules. For example, although light atoms have widely space energy levels, some heavy atoms have their outer orbitals close enough together to give transitions in the visible region. This accounts for the colours of iodides. Transition metals, having partly occupied d or f orbitals, often show absorption bands in the visible region and these are affected by the bonding of ligands e.g Iron(III) reacts with thiocyanate ion to produce an intense red colour due to the iron(III) thiocyanate complex, which may be used to determine iron(III) in the presence of iron(II).

3.2 Instrumentation

The components include (Figure 1.5):

(i) The light sources – a deuterium lamp for the UV region from 190 to 350 nm and a quartz halogen or tungsten lamp for the visible region from 350 to 900 nm.

(ii) The monochromator – used to disperse the light into its component wavelengths, which are further selected by the slit. The monochromator is rotated so that a range of wavelengths is passed through the sample as the instrument scans across the spectrum.

(iii) The optics – may be designed to split the light beam so that the beam passes through two sample compartments, and in such a double-beam instrument, a blank solution can then be used in one compartment to correct the reading or spectrum of the sample. The blank is most commonly the solvent in which the sample is dissolved.

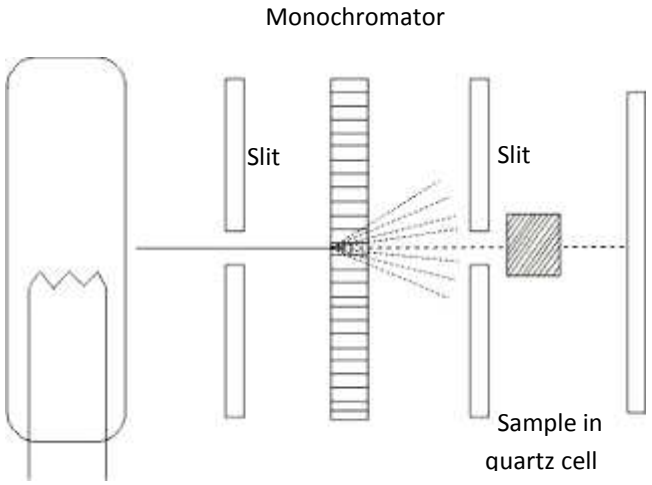


Figure 1.5.
Schematic diagram of a UV/visible Spectrophotometer. . *Reproduced from David G. Watson. (2005) Pharmaceutical Analysis, Elsevier Churchill Livingstone, UK.*

The UV spectrum is usually computer generated in modern UV spectrophotometers and it is characterised by wavy lines with a peak absorbance. Absorbance is plotted on the y axis and wavelength on the x axis (Figure 1.6)

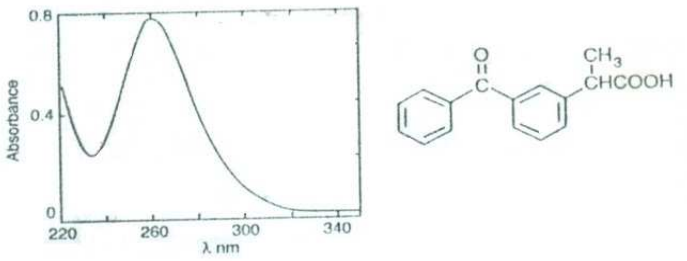


Figure 1.6 A representative UV spectrum (UV spectrum of Ketoprofen). *Reproduced from David G. Watson. (2005) Pharmaceutical Analysis, Elsevier Churchill Livingstone, UK.*

3.3 Factors governing absorption of radiation in the UV/Vis region

pH EFFECTS

pH will affect the structures of compounds with acidic and basic groups, and may cause considerable wavelength shifts

SOLVENT EFFECTS

For π-π^* transitions, the excited state is more polar than the ground state, so it will tend to form dipole-dipole bonds with a polar solvent, such as water or ethanol. This will lower the transition energy and raise the absorption peak wavelength. This is called the red shift (or a bathochromic shift). Tables of solvent corrections are available in specialist texts. For n-π^* transitions, the ground state is often more polar and may form hydrogen or dipole bonds with polar solvents. This increases the transition energy and lowers the peak wavelength shifts causing a blue shift (or hypsochromic shift).

SUBSTITUENT EFFECTS

Substituents that alter the wavelength or absorptivity of a chromophore significantly are called auxochromes. Tables of the effect of substituents plus rules for their application in particular structures are to be found in specialist texts. For example, an unsubstitued unsaturated ketone would have a peak maximum at about 215 nm. Substitution of a hydroxyl group on the carbon next to the carbonyl (α) raises the peak to 250 nm, and two alkyl groups on the next (β) carbons would raise it to 274 nm.

Table 1.1 lists a few of the substituent effects of aromatic compounds. It should be noted that the phenoxide ion (-O-), which is present in alkaline solutions of phenols, absorbs at a considerably longer wavelength than the parent phenol (-OH-). Generally electron donating and lone-pair substituents cause a red shift and more intense absorption. More complex shifts arise when there is more than one substituent present, and tables are given in standard spectrometry texts listings these.

Table 1.1 Absorption maxima for some monosubstituted benzenes Ph-R (in methanol or water)

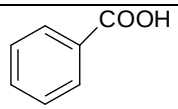
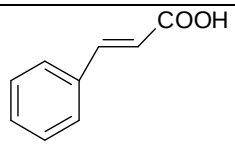
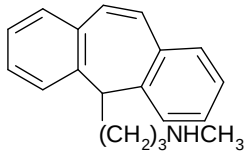
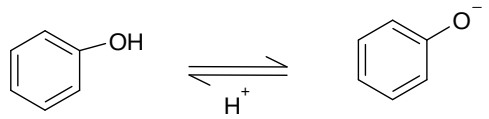
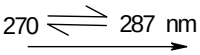
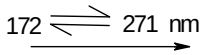
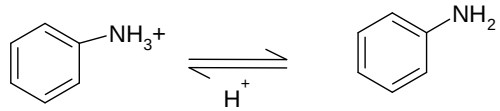
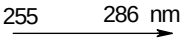
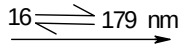
R	Maxima/nm (methanol)	Maxima/nm (water)
-H	204	254
$-CH_3$	207	261
-Cl	210	264
-OH	211	270
$-OCH_3$	217	269
$-CO^{2-}$	224	271
-COOH	230	280
$-NH_2$	230	280
-O-	235	287

STRUCTURE EFFECTS

The structure of organic molecules may be classified in terms of the functional groups, which they contain. Where these absorb UV or visible radiation in a particular region they are called chromophores. Some chromophores important for analytical purposes are listed in Table 1.2. This shows that the absorption by compounds containing only σ bonds such as hexane, or with lone pairs such as ethanol will only occur below 200 nm. These compounds are therefore useful solvents.

If more double bonds are present in a structure in conjugation (i.e. two or more double bonds in a series separated by single bond), absorption takes place at longer wavelengths and with greater intensity. Such extended systems of double bonds are known as ‘chromophores’. The

Table 1.2: The UV absorption characteristics of some chromophores based on the benzene ring.

Chromophore	Longest wavelength λ max (nm)	A (1%, 1cm)
 Benzene	255	28
 Benzoic acid	273	85
 Cinnamic acid	273	1420
 Protriptyline	292	530
 Phenol	 Bathochromic shift	 Hyperchromic shift
 Aniline	 Bathochromic shift	 Hyperchromic Shift

A(1%, 1cm) value, gives a measure of the intensity of absorption. The most common chromophore found in drug molecules is a benzene ring. If the symmetry of the benzene ring is lowered by substitution, the bands in the benzene spectrum undergo a bathochromic

shift – a shift to longer wavelength. Substitution can involve either extension of the chromophore or attachment of an auxochrome (a group containing one or more lone pair of electrons) to the ring and both. The hydroxyl group and amino group auxochromes are affected by pH, undergoing bathochromic (moving to a longer wavelength) and hyperchromic (absorbing more strongly) shifts when a proton is removed under alkaline conditions, releasing an extra lone pair of electrons. The effect is most marked for aromatic amine groups.

Identification of unknown organic samples can be considerably aided by considering the UV-visible absorption spectra. The following general rules may be used as a guide.

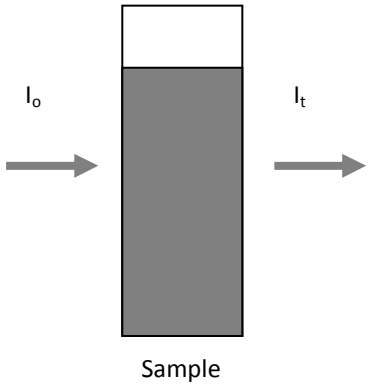
Observation	**Possible conclusion**
No UV absorption present	σ bonds or lone pairs only
Isolated double bonds	
Strong absorption between 200	Aromatic ring
and 250nm (ε ~ 1000)	
Weak absorption near 300 nm (ε ~ 1)	Carbonyl compound

For example, an organic compound, $C_7H_{14}O$ gave a UV spectrum with a peak at 296 nm and ε = 3.7 m^2mol^{-1}. Is it more likely to be a ketone or an alkene? The formula allows the possibility of only one double bond. It must therefore be an alkene with an isolated double bond, absorbing below 200 nm, or a ketone with a weak n-π* transition near 300 nm. The value of both the absorption maximum and of the absorbtivity suggests a ketone.

3.4 Beer-Lambert Law

The measurement of light absorption by a solution of molecules is governed by the Beer-Lambert Law, which is written as follows:

Fig. 1.7.
Absorption of light by a solution.



$\text{Log } I_o/I_t = A = \varepsilon bc$

Where I_o, is the intensity of incident radiation; I_t is the intensity of transmitted radiation; A is known as the absorbance and is a measure of the amount of light absorbed by the sample; ε is

a constant known as the molar extinction coefficient and is the absorbance of a 1M solution of the analyte; *b* is the pathlength of the cell in cm, usually 1 cm; and *c* is the concentration of the analyte in moles litre^{-1}.

Concentration and amounts are usually expressed in grams or milligrams rather than moles and thus for the purposes of analysis of organic molecules, the Beer-Lambert equation is written in the following form:

A = A (1%, 1cm)bc

A is the measured absorbance; A(1%, 1cm) is the absorbance of a 1% w/v (1g/100ml) solution in a 1 cm cell; b is the pathlength in cm (usually 1 cm); and c is the concentration of the sample in g/100 ml. Since measurements are usually made in a 1 cm cell, the equation can be written:

C = $\frac{A}{A(1\%,1cm)}$ which gives the concentration of the analyte in g/100ml.

4.0 Conclusion

In this unit, you have learnt the principles and factors that govern UV/Vis spectroscopy. You will now be familiar with the different parts of the UV spectrophotometer and how to use measurements of the relationship between concentration and absorbance in quantitative analysis using Beer-Lambert Law.

5.0 Summary

- Absorption of UV/Vis radiation occurs through excitation of electrons within the molecular structure to a higher energy state.
- A chromophoric centre is required for UV absorption.
- The UV region is between 200-400 nm, while the visible region is between 400-800 nm.
- A deuterium lamp and quartz halogen lamp is required for UV absorption, while a tungsten lamp is required for the visible region.
- Factors such as pH, solvent, substituent and structure of the compound can affect UV absorption.
- Bathochromic shift is a shift to higher wavelength, while hypsochromic shift is a shift lower wavelength.
- Hyperchromic shift is a shift to stronger absorbance.
- Beer-Lambert Law is obeyed when absorbance is proportional to concentration.

6.0 Tutor-Marked Assignments

1. Draw a diagram showing the different components of a UV spectrophotometer
2. What do you understand by the following terms: (a) chromophore, (b) auxochrome (c) bathochromic shift, (d) hyperchromic shift.
3. Explain how the structure of a molecule can affect the UV absorbance.
4. Explain how solvent and substituents can affect UV absorbance.
5. What is the concentration of the following solutions of drugs in g/100ml and mg/100ml
    i. Carbimazole, A(1%, 1cm) value = 557 at 291 nm, measured absorbance = 0.557 at 291 nm.
    ii. Hydrocortisone sodium phosphate, A(1%, 1cm) value = 333 at 248 nm, measured absorbance = 0.66 at 248 nm.

7.0 References and Other Sources

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed)*. 228-232.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 87-93.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Ltd, Essex, England (1st ed).* 159-163.

4. Robinson J.W., Skelly Frame E.M., Frame G.M. (2005) Undergraduate Instrumental Analysis. *CRC Press Taylor & Francis Group,* New York (6th ed.) 65-80.

UNIT 2: APPLICATIONS OF ULTRAVIOLET/VISIBLE SPECTROSCOPY

1.0 Introduction

The main use of ultraviolet and visible spectroscopy is in quantitative analysis. Many plasma constituents, drugs and other substances are assayed by methods that are based on the measurement of the absorption of a solution of the substance at a specified wavelength in the UV/Vis regions. Other applications include determination of the pKa of a molecule where a pH-dependent UV shift is produced and also for determining the physico-chemical properties of drug molecules prior to formulation and for measuring their release from formulations (dissolution studies).

2.0 Objectives

In this unit, you will learn:

- How UV spectroscopy is used in quantitative analysis of organic molecules
- How it can be used to determine the pKa of a molecule
- How UV spectroscopy can be used for determining the physic-chemical properties of drug molecules prior to formulation and also drug dissolution studies.

3.0 Main Content

3.1 Applications of UV/Visible spectroscopy to quantitative analysis

Many organic compounds and inorganic complexes may be determined by direct absoptiometry using the Beer-Lambert Law. The calculation of the concentration is made either by direct substitution of the appropriate quantities in the equation for the Beer-Lambert law if the compound obeys the law or by plotting a calibration curve of the concentrations of solutions of known strength against their absorbance at a specified wavelength and reading the concentration of the unknown solution from the graph after measuring its absorbance (Figure 1.8) The latter can be carried out automatically by the spectrometer, which can also include an internal standardisation routine. Even if the compound does not strictly obey the Beer-Lambert law the concentration of a solution can still be obtained using a calibration curve provided a sufficient number of points are plotted.

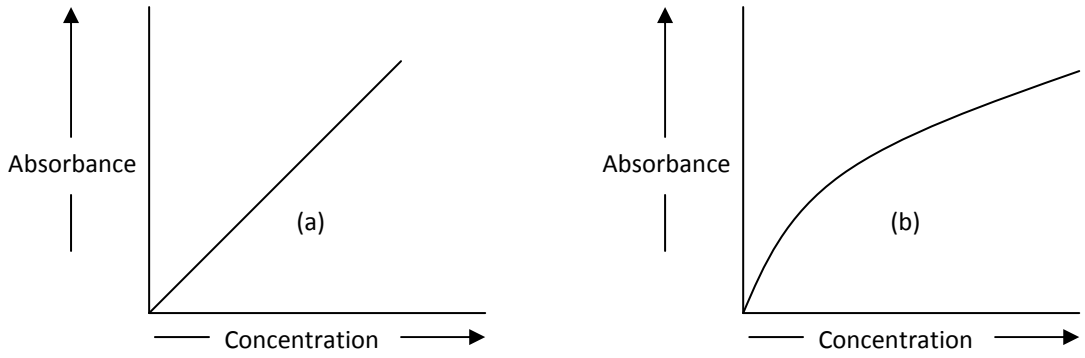


Fig. 1.8. Typical calibration curves. The Beer –Lambert law is obeyed in (a) but not in (b).

It is important to recognize that for most accurate work or determination of trace amounts, three criteria must be observed.

i) The absorptivity of the species to be determined must be reasonably large. While it is possible to determine metals such as copper or cobalt in water as the aquo complex, this will give accurate results only down to about 1% since $\varepsilon \sim 10 m^2 mol^{-1}$. However, for anthracene, $C_{14}H_{10}$, which has three fused aromatic rings, $\varepsilon = 18\ 000 m^2\ mol^{-1}$ and thus, even a solution of about 0.5 ppm will give an absorbance of approximately 0.1 in a 1 cm cell.

ii) These species must be stable in solution. It must not oxidize or precipitate or change during the analysis (unless the analysis intends to study that change).

iii) Calibration must be carried out over the range of concentrations to be determined. Agreement with the Beer-Lambert law must be established.

iv) In complex matrices, it is not possible to analyze for all the species present using a few spectra. It is necessary to separate the components using a chromatographic technique.

It should be noted that it is possible to determine two (or more) species in an analytical sample by measuring the absorbance at several wavelengths. Calibration and measurements at two wavelengths enables two components to be determined simultaneously, but if more wavelengths are measured, a better 'fit' of the experimental data is achieved.

Example

Two organic components X and Y have absorption maxima at 255 and 330 nm, respectively.

For a pure solution of X, $\varepsilon(255) = 4.60$; $\varepsilon(330) = 0.46$

For a pure solution of Y, $\varepsilon(255) = 3.88$; $\varepsilon(330) = 30.0$

For a mixture of X and Y in a 0.01 m cell, A (255) = 0.274 and A(330) = 0.111

Calculate the concentrations of X and Y in the mixture.

Using the Beer-Lamber law at each wavelength:

$A = \varepsilon_x c_x l + \varepsilon_y c_y l$

At 255nm: $0.274/0.01 = 4.60c_x + 3.88c_y$

At 330 nm: $0.111/0.01 = 0.46c_x + 30.0c_y$

Solving these simultaneous equations gives

$C_x = 5.71$ mol $m^{-3} = 5.71 \times 10^{-3}$ M

$C_y = 0.288$ mol $m^{-3} = 2.88 \times 10^{-4}$ M

3.1.1 Applications of UV/visible spectroscopy in Pharmaceutical quantitative analysis.

The pharmaceutical industry rely heavily on simple analysis by UV/visble spectrophotometry to determine the active ingredients in formulations. These methods are usually based on the use of standard A(1%, 1cm) value for the active ingredient being assayed and this relies on the instrument being accurately calibrated. There should be no interference from excipients (preservatives, colorants etc) present in the formulations and the sample should be free of suspended matter, which could cause light scattering.

Example:

A typical example of a straightforward assay is the analysis of furosemide tablets:

i. Tablet powder containing ca. 0.25 g of furosemide (frusemide) is shaken with 300ml of 0.1 M NaOH to extract the acidic furosemide (frusemide).
ii. The extract is then made up to 500 ml with 0.1 M NaOH.
iii. A portion of the extract is filtered and 5 ml of the filtrate is made up to 250 ml with 0.1M NaOH.
iv. The absorbance of the diluted extract is measured at 271 nm.
v. The A(1%, 1cm) value at 271 nm is 580 in basic solution.

From the data below calculate the % of stated content in a sample of furosemide tablets:

- Stated content per tablet; 40 mg of furosemide (frusemide)
- Weight of 20 tablets: 1.656 g
- Weight of tablet powder taken for assay: 0.5195 g
- Absorbance reading; 0.596

Calculation:

Expected content in tablet powder taken: $\frac{0.5195}{1.656}$ x 40 x 20 = 251.0 mg

Concentration in diluted tablet extract: $\frac{0.596}{580} = 0.001028 \frac{g}{100} ml = 1.028\ mg/100\ ml$

Concentration in original tablet extract: 1.028 x 50 = 51.40 mg/100 ml.

Volume of original extract: 500 ml.

Therefore, amount of furosemide (frusemide) in original extract; 51.40 x 5 = 257.0.

Percentage of stated content: $\frac{257.0}{251.0} \times 100 = 102.4\%$

3.2 Application of UV/Visible spectroscopy in the determination of pKa values

It is possible to use UV/Vis spectroscopy to determine the pKa of the ionisable group responsible when a pH-dependent UV shift is produced. In the case of phenylephrine, the pKa value of the phenolic group can be determined conveniently from the absorbance at 292 nm, since the absorbance of the molecular species where the phenolic group is un-ionised is negligible at this wavelength. This is not the case for all molecules. A general equation for determination of pKa from absorbance measurement at a particular wavelength is given below. The following equation can be used for an acid (for a base the log term is subtracted) where increasing pH produces a bathochromic/hyperchromic shift:

$$pKa = pH + \log \frac{Ai - A}{A - Au}$$

Where A is the measured absorbance in a buffer of known pH at the wavelength selected for analysis; Ai is the absorbance of the fully ionised species; and Au is the absorbance of the un-ionised species.

The wavelength used for analysis is one where there is great difference between the ionised and un-ionised species. An approximate knowledge of the pKa value is required to select a suitable pH value, within ± 1 of the pKa value, for measurement of A. For accurate determination, measurement is made at a number of closely spaced pH values. It should be noted that if the acid or base undergoes a shift to lower absorbance and shorter wavelength with increasing pH the log term above is subtracted; this situation is less common in drug molecules.

Example:

The absorbance of a fixed concentration of phenylepherine at 292 nm is found to be 1.224 in 0.1M NaOH and 0.02 in 0.1M HCl. Its absorbance in buffer at pH 8.5 is found to be 0.349. Calculate the pKa value of its acidic phenolic hydroxyl group.

$pKa = 8.5 + \log \frac{1.224 - 0.349}{0.349 - 0.02} = 8.5 + 0.402 = 8.902$

3.3 Application of UV/Visible spectroscopy in Preformulation and Formulation of Drugs

Physico-chemical properties of drug molecules prior to formulation and studying of the release of drugs from formulations can be determined by UV/Vis spectroscopy. The type of properties which can be usefully determined by UV method are as follows:

Partition Coefficient

The partition coefficient of a drug between water and an organic solvent may be determined by shaking the organic solvent and the water layer together and determining the amount of drug in either the aqueous or organic layer by UV spectroscopy. If buffers of different pH values are used, the variation of partition coefficient with pH may be determined and this provides another means of determining the pKa value of a drug.

Solubility

The solubility of a drug in, for instance, water may be simply determined by shaking the excess of the drug in water or buffer until equilibrium is reached and then using UV spectroscopy to determine the concentration of the drug that has gone into solution. Solubility of an ionisable group present in the drug can be determined by dissolving varying concentrations of the salt of the drug in water and then adding excess acid to a solution of the salt of an acidic drug or excess base to a solution of the salt of a basic drug, thus converting the drugs into their un-ionised forms. When the solubility of the un-ionised drug in water is exceeded, a cloudy solution will result and UV spectrophotometry can be used to determine its degree of turbidity by light scattering, which can be measured at almost any wavelength e.g. 250 nm.

Release of a drug from a formulation

UV spectrophotometry is routinely used to monitor in vitro release of active ingredients from formulations (drug dissolution studies).

3.4 Identification of chromophores in qualitative analysis

UV and visible spectra are used to identify chromophores in qualitative analysis. Identification is carried out by comparing the spectrum of the unknown compound to those of known chromophores by consulting suitable source books, such as Organic Electronic Spectral Data (published by Wiley). If, within experimental error, the spectrum of the unknown compound matches that of a chromophore in the source book, it is taken as evidence that the chromophore is found in the structure of the unknown compound. The process is similar to that used to identify a person from their fingerprints. The procedure follows no set rules and it is largely a matter of experience.

4.0 Conclusion

In this unit you have learnt the different applications of UV spectroscopy in quantitative analysis in particular pharmaceutical quantitative analysis. You have also learnt how this

technique can be used to determine the pKa of an organic molecule and the use in pre-formulation and dissolution studies of drug molecules.

5.0 Summary

- The concentration of a sample can be determined using the Beer-Lambert equation if the A1% value is known.
- Calibration curve of a solution of known concentration can be used to determine the concentration of an unknown solution.
- Two or more components in a sample can also be resolved.
- Pharmaceutical analysis rely heavily on UV spectroscopy as most drug molecules have chromophores, hence used in drug assays.
- The pKa, solubility and partition coefficient of a molecule can also be determined by UV spectroscopy.
- Physico-chemical properties of a drug as well as drug release from the formulation (dissolution studies) can also be studied by this technique.

6.0 Tutor-Marked Assignment (TMA)

1. Discuss two different applications of UV spectroscopy.
2. Calculate the pKa value of the weakly basic aromatic amine in procaine from the data given below. Absorbance of a fixed concentration of procaine in 1 M HCl at 296 nm = 0.031; absorbance in buffer at pH 2.6 = 0.837.
3. Calculate the percentage of stated content of promazine hydrochloride in promazine tablets from the following information:
    i. Tablet powder containing ca. 80 mg of promazine hydrochloride is ground to a paste with 10 ml of 2 M HCl.
    ii. The paste is then diluted with 200 ml of water, shaken for 15 min and finally made up to 500 ml.
    iii. A portion of the extract is filtered.
    iv. 5 ml of the filtrate is taken and diluted to 100 ml with 0.1 M HCl.
    v. The absorbance is read at a wavelength of 251 nm.

- A(1%,1 cm) value of promazine.HCl at 251 nm = 935
- Stated content of promazine. HCl per tablet = 50 mg.
- Weight of 20 tablets = 1.667 g
- Weight of tablet powder taken for assay = 0.1356 g.
- Absorbance reading = 0.755.

7.0 References/Further Readings

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed)*. 228-232.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 87-111.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Ltd, Essex, England (1st ed).* 159-163.

UNIT 3: INFRARED SPECTROSCOPY

1.0 Introduction

Vibrational transitions in molecules cause absorption in the infrared region of the electromagnetic spectrum. All substances absorb infra-red radiation. A simple explanation of this absorption process can be obtained by picturing the molecules forming the substance as solid balls representing the atoms linked by springs representing bonds. This structure will be in a state of perpetual wobbly motion, this movement taking the form of the bonds stretching, contracting, bending, twisting etc. The energy of the absorbed radiation is dissipated within the molecule by increasing the intensity of this molecular movement. It is possible to relate the wavelength of the absorption to a specific bond.

2.0 Objective

You will learn:

- The principles of IR spectroscopy
- The difference between near, far and middle IR regions of the electromagnetic radiation.
- About the instruments used to measure IR absorption
- About the IR spectrum and interpretation

3.0 Main Content

3.1 Principles of Infrared Spectroscopy

Electomagnetic radiation ranging between 400 cm^{-1} and 4000 cm^{-1} (2500 and 20 000 nm) is passed through a sample and is absorbed by the bonds of the molecules in the sample causing them to stretch and bend. The wavelength of the radiation absorbed is characteristic of the bond absorbing it.

The infra-red region can be divided up as shown in table 1.3 below:

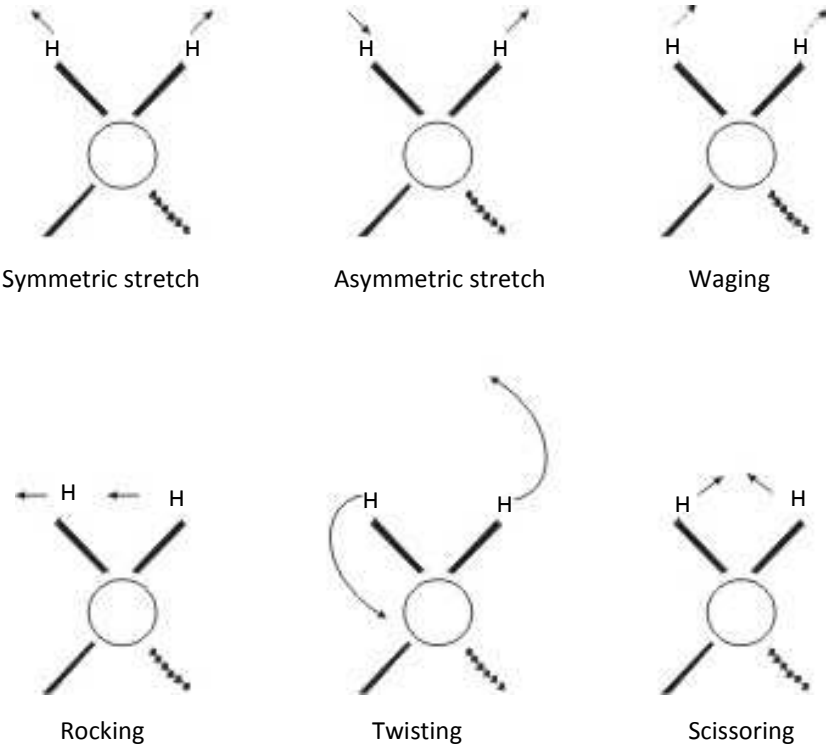
Table 1.3 The Infra-red Ranges

Ranges	**Far Infrared**	**Middle Infrared**	**Near Infrared**
Wavelength range	50-1000 µm	2.5-50µm	0.8-2.5µm
Wave number range	200-10 cm^{-1}	4000-200 cm^{-1}	12 500-4000 cm^{-1}
Energy range	0.025-0.0012 eV	0.5-0.025 eV	1.55-0.5 eV

The middle infra-red range is commonly used for structural confirmation, but near-infrared spectrophotometry, which has been used for many years to control products such as flour and animal feed, is finding increasing applications in quality control in the pharmaceutical industry. The rules governing transitions in the infrared region of the spectrum requires that, in order to absorb, the dipole moment of the molecule must change during vibration. Such vibrations are said to be IR active.

In order for the electrical component in electromagnetic radiation to interact with a bond, a bond must have a dipole. Thus symmetrical bonds, such as those in O_2 or N_2, do not absorb infrared radiation. The electrical field associated with electromagnetic radiation will interact with the molecule to change its electrical properties. Some molecules (e.g. HCl) have a dipole moment due to charge separation and will interact with the field. Others may acquire a dipole when they vibrate. For example, methane, CH_4 has no dipole, but when one of the CH bonds stretches, the molecule will develop a temporary dipole. Even if the molecule does not have a dipole, the electric field, E, may distort the electron distribution and polarize the molecule. Majority of organic molecules have plenty of asymmetry. Even in small organic molecules the modes of vibration are complex. This is illustrated by the vibrational modes which can occur in methylene group (Figure 1.9) The large number of bonds in polyatomic

Fig. 1.9.
Vibration modes of a methylene group.



molecules mean that the data obtained by IR analysis is extremely complex and provides a unique 'fingerprint' identity for the molecule. Quite a lot of structural information can be obtained from an IR spectrum, but even with modern instrumentation it is not possible to completely 'unscramble' the complex absorbance patterns present in IR spectra.

3.2 Factors determining the intensity and energy level of absorption in IR-Spectroscopy

Intensity of absorption

a.The intensity with which a bond absorbs radiation depends on its dipole moment. Thus the order of intensity of absorption for the following C-X bond is :

C-O > C-Cl > C-C-OH > C-C-H

Similarly:

OH > NH > CH

b.The intensity depends on the relative electronegativity of the atoms involved in the bond.

c. The intensity of the stretching of carbon-carbon double bonds is increased when they are conjugated in a polar double bond. The order of intensity is as follows:

C=C-C=O > C=C-C=C > C=C-C-

Energy level of absorption

The equation which determines the energy level of vibration of a bond is shown below:

$$E_{vib} \propto \sqrt{k/\mu}$$

k is a constant related to the strength of the bond, e.g. double bonds are stronger than single bonds and therefore absorb at a higher energy than single bonds. μ is related to the ratio of the masses of the atoms joined by the bond.

$$\mu = \frac{m1m2}{m1+m2}$$

e.g for O-H bonds, $\mu = \frac{16 \times 1}{17} = 0.94$; for C-O bonds, $\mu = \frac{12 \times 16}{28} = 6.86$

Where m_1 and m_2 are the masses of the atoms involved in the bond. According to the μ term, the highest energy bonds are the X-H (OH, NH, CH) The order of energy absorption for some common bonds is as follows, which reflects μ and the strength of the bonds:

O-H > N-H > C-H > C≡C > C=O > C=C > C-F > C-Cl

3.3 Instrumentation

Two types of instrument are commonly used for obtaining IR spectra: dispersive instruments, which use a monochromator to select each wavenumber in turn in order to monitor its intensity after the radiation has passed through the sample, and Fourier transform instruments, which uses an interferometer. The latter generates a radiation source in which individual wavenumbers can be monitored within a *ca* 1 s pulse of radiation without dispersion being required. In recent years, Fourier transform instruments have become very common. A simple diagram of continuos wave instrument is shown in Figure 1.10 . The actual arrangement of the optics is much more complicated than this but the diagram shows the essential component parts for a dispersive IR instrument. The filament used is made of metal oxides e.g zirconium, yttrium and thorium oxides and is heated to incandescence in air.

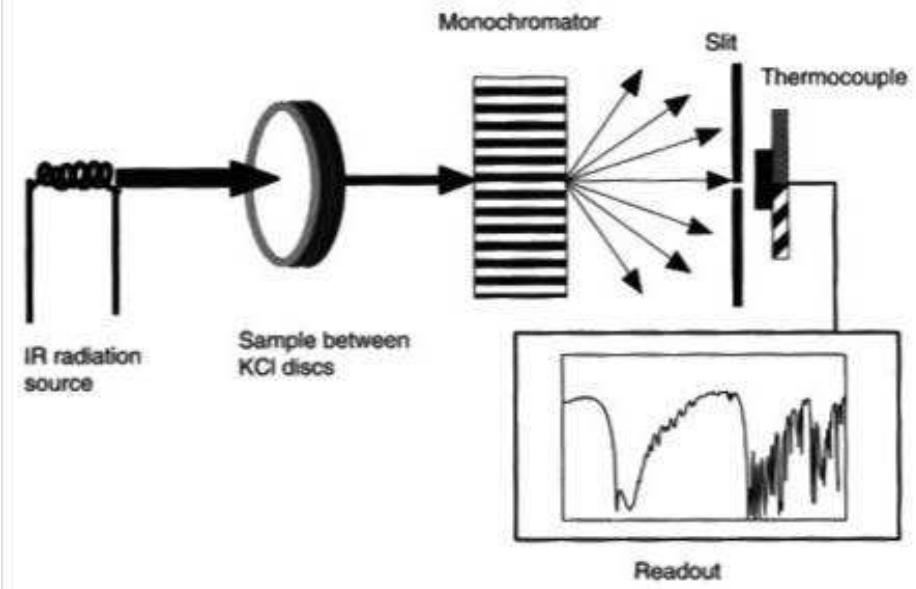


Figure. 1.10. Schematic diagram of a continuous wave IR instrument. *Reproduced from David G. Watson. (2005) Pharmaceutical Analysis, Elsevier Churchill Livingstone, UK.*

The sample is contained in various ways within discs or cells made of alkali metal halides. Once the light has passed through the sample, it is dispersed so that an individual wavenumber or small number of wavenumbers can be monitored in turn by the detector across the range of the spectrum.

The principles are the same in a Fourier transform IR instrument except that the monochromator is replaced by an interferometer. An interferometer uses a moving mirror to displace part of the radiation produced by a source (Figure 1.11), thus producing an interferogram,

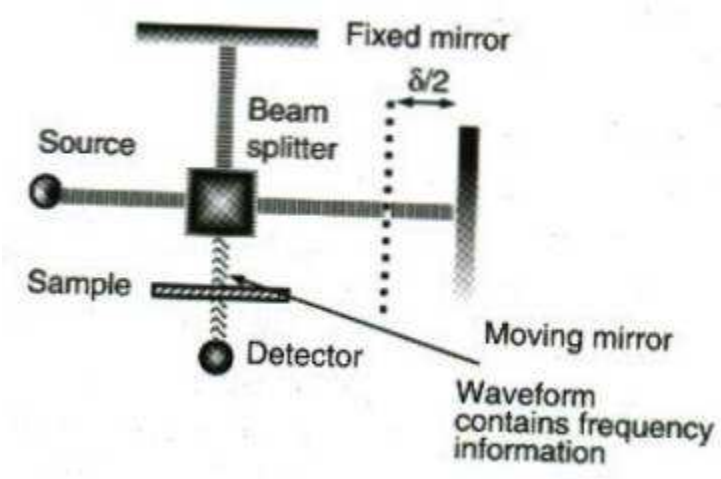


Fig. 1.11
Michelson Interferometer used in FT-IR instruments. *Reproduced from David G. Watson. (2005) Pharmaceutical Analysis, Elsevier Churchill Livingstone, UK.*

which can be transformed using an equation called the 'Fourier transform' in order to extract the spectrum from a series of overlapping frequencies. The advantage of this technique is that a full spectral scan can be acquired in about 1 s, compared with the 2-3 min required for a dispersive instrument to acquire a spectrum. Also, because the instrument is attached to a computer, several spectral scans can be taken and averaged in order to improve the signal:noise ratio for the spectrum.

Sample preparation

Gases

Gases are usually present in lower concentrations compared to pure solids and liquids (e.g. 0.04 M for nitrogen in air, 17.4 M for liquid ethanol), longer path lengths are required. The gas-phase spectrum of HCl at 0.2 atm may be studied in a 10 cm glass cell with NaCl windows. Low concentrations of exhaust gases may need a 10 m cell, which reflects the IR beam to achieve the long pathlength.

Liquids

These are more concentrated and may be studied directly as a thin film between NaCl plates. For more quantitative work, accurately prepared solutions in solvents that do not absorb in the region of analytical interest such as CCl_4 or CS_2 in NaCl cells, with a known path length provided by a space, may be used. Most of these are also applicable to NIR, and short path length silica cells may also be used.

Solids

If a solid organic powdered sample is placed in an IR beam, the particles scatter the light, and light is transmitted. Therefore, for routine analysis, the sample is usually ground to a fine powder and mixed with paraffin oil (Nujol) to form a paste or mull. This reduces the scattering at the powder surface and gives a food spectrum, with the disadvantage that the bands due to the oil (at approximately 2900, 1450, 1380, 750 cm^{-1}) are superimposed on the spectrum. Alternatively, the fine powder KBr and the mix pressed in a hydraulic press between smooth stainless steel dies to give a clear KBr disk. Solutions of solids may also be used and tetrachloromethane, CCl_4, is often used as solvent, since it has few IR-active bands,

mostly at the low wavenumber end of the spectrum. These must be ignored when the spectrum is interpreted. Thin films of solids such as polymers may be supported directly in the IR beam. Polystyrene is a useful calibration sample to check the performance of an IR spectrometer.

A more recent development in sample preparation is the use of reflectance spectra.

Reflectance Spectra

Reflectance spectra can be measured in three ways. A powder placed in the incident beam and allowed to interact by diffuse reflectance. The reflections are collected by a mirror. If the beam is reflected off a flat sample surface, specular reflectance results and this may give a good spectra.

If the sample is placed in good contact with the surface of an optical device of high refractive index (such as a prism of KRS-5) and illuminated through the prism by IR, the beam passes into the layers in contact and is attenuated before being totally internally reflected by the system. This is called attenuated total reflectance or ATR. If the beam interacts several times, then we have multiple internal reflectance (MIR) and if the surface is horizontal, which is an advantage in setting up the sample, then it is horizontal attenuated total reflectance (HATR).

It should be noted that the detail of spectra obtained by reflectance methods might be different from that obtained in solution or with KBr disk techniques. Modern instruments possess software to convert reflectance spectra to resemble the more usual transmission spectra.

Analysts often deal with samples of very small size or analyze a small area of a large sample. One technique is to reduce the size of the IR beam using a beam-condensing accessory. A more versatile modern development is the IR microscope.

3.4 The Infra-red Spectrum

The infra-red spectrum of a compound is usually presented as a plot of transmittance against wavenumber, the reciprocal wavelength. Absorptions are recorded as downward peaks. (The spectrum is normally taken using a dilute solution of the compound in a suitable non-aqueous solvent or a solid solution in potassium bromide or a nujol mull. Spectra may also be taken in the form of liquid films and the vapour state. These different sampling techniques can affect the appearance of the spectrum of a particular compound. Spectra taken in non-polar solvents such as tetrachlormethane and alcohol free trichloromethane are preferred since fewer intermolecular forces are found in these solutions. As a result, the resolution of the spectrum will be better, that is, it will have sharper and better defined peaks. Intermolecular forces such as hydrogen bonding, tends to broaden the absorption peaks and in some cases the result is a broad absorption band rather than a narrow peak in the spectrum.

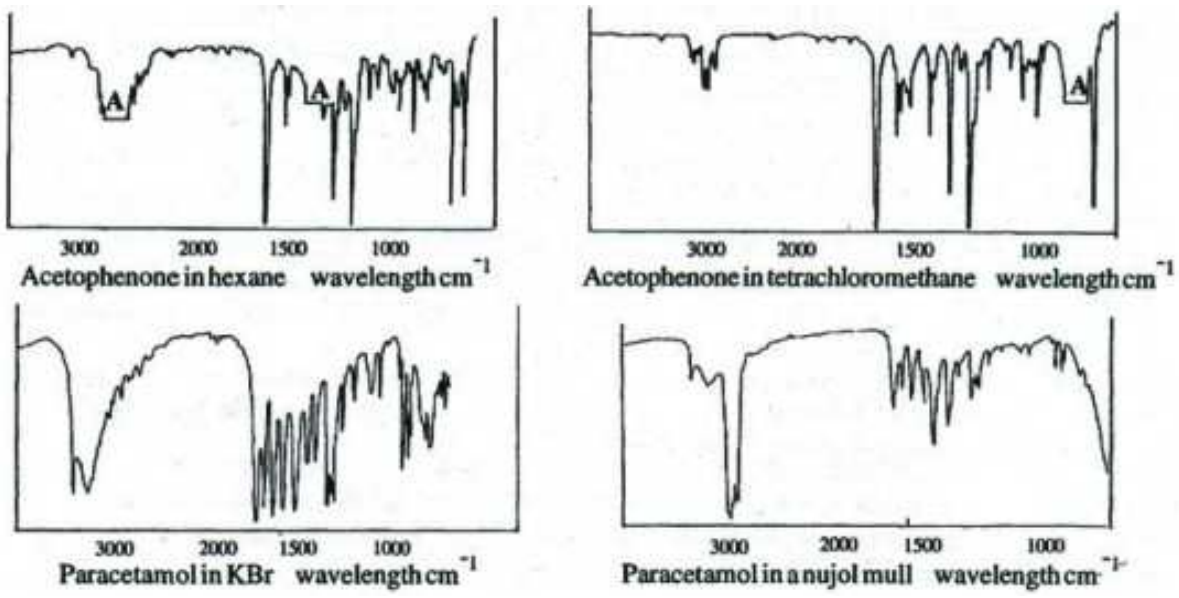


Fig. 1.12. The infra-red spectra of acetophenone and paracetamol recorded using different solvents. Note the differences between the spectra of each compound and also the 'dead pan peaks' (A) where the interaction of the absorptions of the solvent and compound results in no signal reaching the recorder's pen. Note also the peaks due to the nujol at 2850-2950 (strong), 1460 and 1370 cm^{-1} (weak). *Reproduced from G. Thomas. (1996) Chemistry for Pharmacy and the Life Sciences, Pearson Education Ltd, Essex, England*.

3.5 Interpretation of Infrared Spectra

Many functional groups absorb at characteristic wavelengths in the infra-red region of the electromagnetic spectrum. The positions of these absorptions show little variation with change in molecular environment and so can be used in reverse to identify the presence of a functional group in a molecule. The initial interpretation of a spectrum is made using correlation tables from textbooks. These tables are of a general nature and are not likely to have been compiled under the same conditions that an investigator would use to run a spectrum in the laboratory. A functional group may give rise to a peak in the spectrum that is at a significantly different wavenumber from that recorded for that structure in the correlation table. This must be borne in mind when interpreting spectra.

It is not feasible to interpret all the peaks in a spectrum form tables of this type but with practice it is possible to pick out the key ones and relate them to functional groups within the molecule. Carbonyl groups for example, exhibit strong absorptions in 1600 to 1780 cm-1 region. Further examination of the spectrum may enable one to speculate further on the exact nature of this carbonyl group. Aldehydes, for example, have a C-H stretching absorption at about 2700 to 2900 cm-1; ketones and esters do not absorb in this region whilst acids and amides have broad O-H stretching absorption bands in the 2700 to 3600 cm-1 region. Many functional groups can be detected by this method but deductions of this nature should be backed up by other evidence such as chemical tests and other forms of spectroscopy. A more detailed interpretation of a spectrum can be obtained by consulting specialised tables of absorptions for the particular type of structure being studied.

4.0 Conclusion

You will now understand the principles of IR spectroscopy and the factors that govern the absorption and energy level of absorption in the IR region. The difference between the continuous wave and FT-IR instruments have been discussed and you now know what an IR spectrum looks like and how it can be interpreted.

5.0 Summary

- All molecules absorb IR radiation.
- Vibrational transitions in molecules cause absorption in the infra-red region of the electromagnetic radiation.
- The middle infra-red range is commonly used for structural confirmation, though near-infrared is now finding increasing use.
- Solid samples are usually prepared mixed with nujol or as KBr discs.
- Liquid samples can be studied directly as a thin film between NaCl discs.
- The infra-red spectrum is presented as a plot of transmittance against wavenumber.
- Functional groups absorb at characteristic wavelengths.

6.0 Tutor-Marked Assignments

1. Discuss the principles of IR spectroscopy.
2. Highlight the differences between a continuous wave IR spectrometer and the FT-IR instruments.
3. Discuss factors that determine the intensity and energy level of absorption in IR-spectroscopy.

7.0 References/Further Readings

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed)*. 233-241.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 114-134.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 166-169

UNIT 4 APPLICATIONS OF INFRA-RED SPECTROSCOPY

1.0 Introduction

Infra-red spectroscopy is mainly used for qualitative analysis in structure elucidation, fingerprinting, following the course of a reaction and in the identification of Polymorphs. Use in quantitative analysis is less accurate than other analytical methods and is seldom used.

2.0 Objectives

In this unit, you will learn:

- How IR spectroscopy can help in identifying the functional groups present in a molecule.
- The use of IR spectroscopy in structure elucidation.
- The fingerprint technique in identifying an unknown compound.
- How to monitor a reaction with IR spectroscopy and how the technique can be used in quantitative analysis
- The use of IR spectroscopy in identifying polymorphs.

3.0 Main Content

3.1 Structure elucidation

The extent to which IR spectroscopy is used in structure elucidation is limited. The information that can be obtained is limited to recognizable bands in the IR spectra of molecules. The most readily assigned absorptions are usually at >1500cm^{-1}.

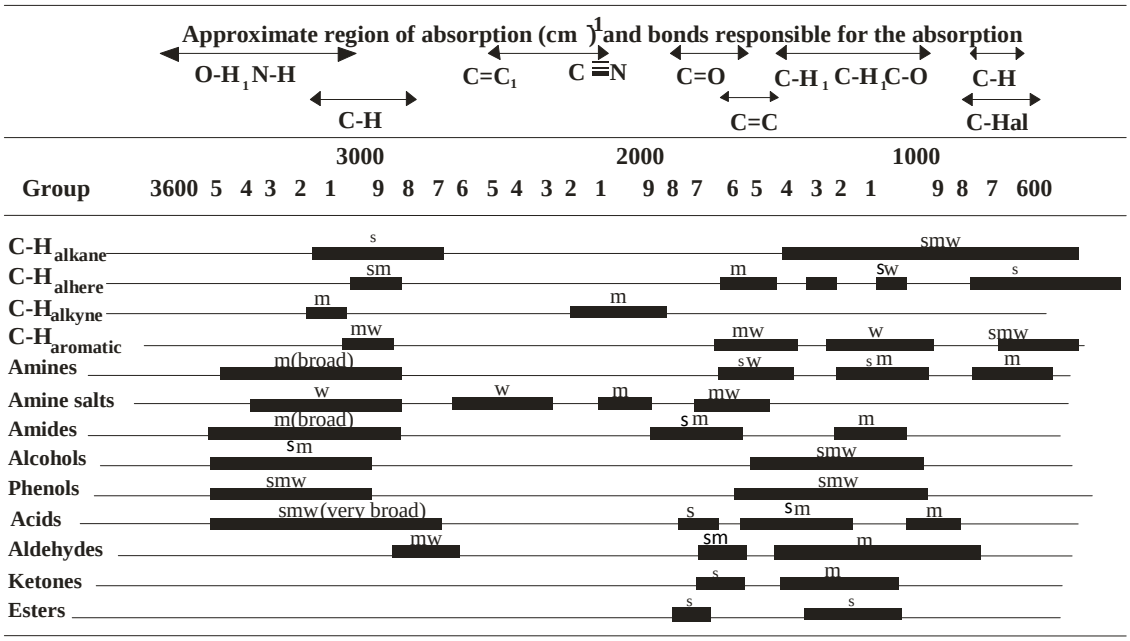


Figure 1.13: An example of an infra-red, correlation chart. The letters s, m, and w indicate that the absorptions are usually either strong, medium or weak. Groups of letter indicate that the peaks may have different

strengths. Note m' refers to peaks due to conjugated C=C bonds only. *Reproduced from G. Thomas. (1996) Chemistry for Pharmacy and the Life Sciences, Pearson Education Ltd, Essex, England.*

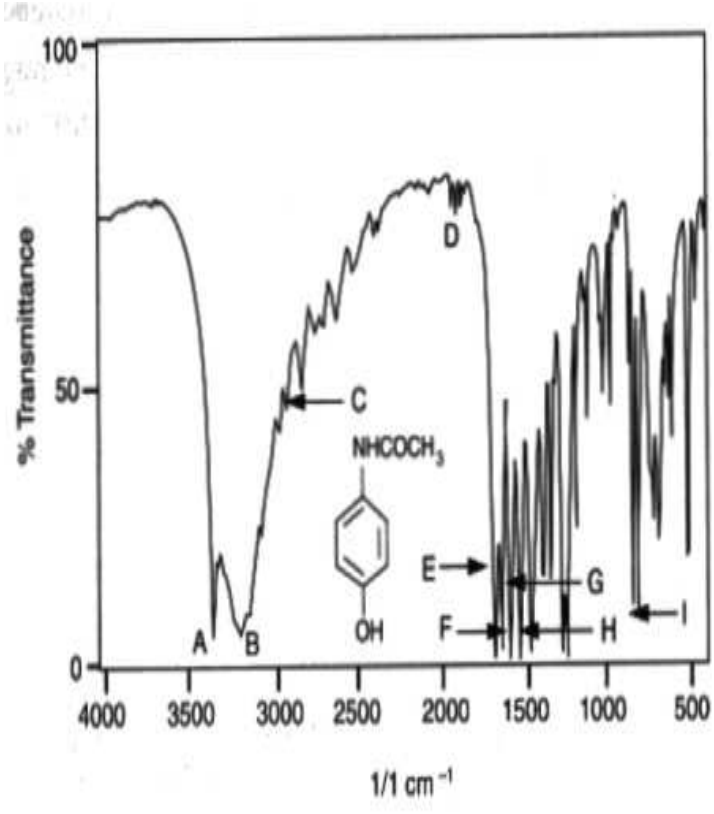


Figure 1.14: Infra-red spectrum of Paracetamol as a KBr disc. *Reproduced from David G. Watson. (2005) Pharmaceutical Analysis, Elsevier Churchill Livingstone, UK.*

3.2 Infrared Spectroscopy as a fingerprint technique

In practice, it is difficult to correlate absorptions with functional group structures in the region below 1500cm^{-1}. This area, because of its complexity, is known as the fingerprint region. It is particularly useful for identifying an unknown compound by comparing its spectrum with the spectra of known compounds recorded under the same conditions. If the spectrum of the unknown matches that of a known compound it is probably the same compound. This procedure is known as fingerprinting. Deductions made by infra-red fingerprinting should be supported by additional evidence.

Table 1.4: Interpretation of the IR spectrum of Paracetamol

Wavenumber	Assignment	Comments
A 3360 cm-1	N-H amide stretch	This band can be seen quite clearly although it is on top of the broad OH stretch
B 3000-3350 cm-1	Phenolic OH stretch	Very broad due to strong hydrogen bonding and thus obscures other bands in this region
C *ca* 3000 cm-1	C-H stretching	Not clear due to underlying OH absorption
D 1840-1940 cm-1	Aromatic overtone region	Quite clear fingerprint but does not reflect 2 band pattern proposed for p-di-substitution
E 1650 cm-1	C=O amide stretch	C=O stretching in amides occurs at a low wavenumber compared to other unconjugated C=O groups
F 1608 cm-1	Aromatic C=C stretch	This band is strong since the aromatic ring has polar substituents which increase the dipole moment of the C=C bonds in the ring.
G 1568 cm-1	N-H amide bending	Strong absorption in this case but this is not always so
H 1510	Aromatic C=C stretch	Evidence of a doublet due to interaction with ring substituents
I 810 cm-1	=C-H bending	Possibly aromatic C-H bending but the fingerprint region is too complex to be completely confident of the assignment.

3.3 Identification of Polymorphs

IR spectroscopy along with differential scanning calorimetry and X-ray powder diffraction provides a method for characterising polymorphic forms of drugs. The existence of polymorphs, different crustalline forms of a substance has an important bearing on drug bioavailabiliy, the chemical processing of the material during manufacture and on patent lifetime. Until recently the standard method of sample preparation for characterising polymorphs by IR was by using a Nujol mull to prepare the sample. However, the DRIFT technique has an advantage since it does not introduce interfering peaks which are present in Nujol and which may obscure areas of interest in the fingerprint region of the spectrum. In addition, low polarity samples may be soluble in Nujol, thus causing their polymorphs to breakdown.

3.4 Reaction Monitoring

Infra-red spectroscopy is used to follow reactions by observing the disappearance and appearance of relevant peaks, for example, the synthesis of ethyl benzoate from benzonitrile (Fig 1.15). However, samples must be isolated from the reaction for accurate analysis.

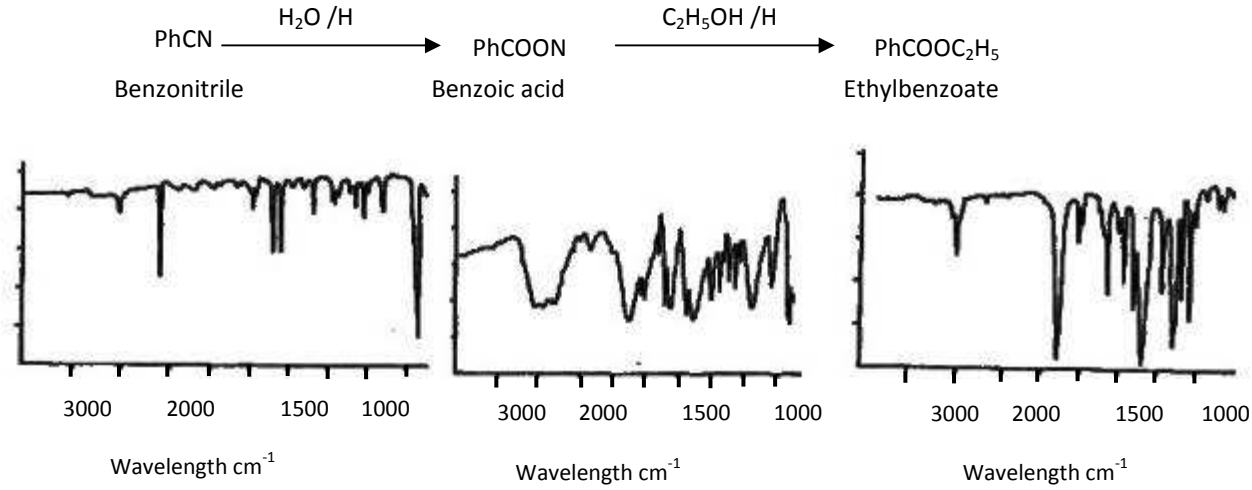


Figure 1.15: Monitoring of the course of reaction for the Synthesis of ethylbenzoate by infra-red spectroscopy. *Reproduced from G. Thomas. (1996) Chemistry for Pharmacy and the Life sciences. Pearson Education Ltd., Essex, England.*

In Figure 1.15 above, the first step, the sharp peak at 2228 cm^{-1} due to the nitrile disappears and a broad peak due to the absorption of the O-H bond of the carboxylic acid appears. In step two the broad carboxylic acid peak is replaced by a narrow peak due to aromatic C-H bond absorption whilst the peak at 1682 cm^{-1} due to the carbonyl group of the carboxylic acid is replaced by a narrow peak at 1718 cm^{-1}, due to the carbonyl group of the carbonyl group of the ester. It should be noted that although the spectra in this figure are run in different

solvent systems, it is still possible to use them to follow the synthesis. However, due allowances must be made if different solvents are used when following a synthesis.

3.5 Quantitative Analysis

Infrared spectroscopy is seldom use for quantitative analysis as less accurate than other analytical methods. However, it does have one advantage over ultra-violet and visible spectroscopy. The large number of well defined absorption peaks means that it is possible to assay the individual components of a mixture provided a peak that is due only to the substance being assayed (analyte) can be found. For example, automated quantitative infra-red spectroscopy has been used to determine the concentrations of contaminants, such as carbon monoxide, chloroform and methanol in air. However, it can be difficult to locate peaks due solely to the analytes in an infra-red spectra of a mixture.

In order to make quantitative measurements, it is necessary to convert the transmittance readings to absorbance, A, the relation between the two being:

$$A = \log (100/T\%)$$

This allows any absorbance by solvents or other components of the sample to be subtracted form the analyte peak.

$$A(\text{total}) = A (\text{sample}) + A (\text{background})$$

This allows the proper subtraction of solvents or other components. For example, if the spectrum of a machine oil without additives is measured in a 0.1 mm NaCl cell, and then the same procedure is followed for a sample with small amounts of additives, subtraction of the absorbance spectra will give the spectrum of the additives in absorbance form.

Gas analysis by IR spectrometry using long path length cells has been used to measure concentrations of anaesthetic gases. For example, nitrous oxide, N_2O, shows a strong absorbance at 2200 cm^{-1} at which the wavenumber of neither water vapour nor carbondioxide interfere. Measurement of the concentrations between 2 and 50 ppm is possible with a 15 m path length gas cell, Trichloromethane (chloroform) gives a strong, sharp peak at 770 cm^{-1} and may be measured down to 0.1 ppm.

IR spectroscopy has been used to measure the mineral contents of rocks, asbestos and to study residual solvents in Pharmaceuticals. Mixtures can be analyzed directly, although chromatographic methods are normally preferred.

4.0 Conclusion

You would have learnt the different applications of IR spectroscopy, in particular its use in identifying the functional groups in a molecule.

5.0 Summary

- The IR spectrum is a good indicator of the molecular structure of a compound.
- IR spectrum can be complex making interpretation very difficult.
- The fingerprint region is below 1500cm^{-1}.
- Fingerprinting technique can be used in identifying an unknown compound.
- IR can be used to monitor a reaction by observing the appearance or disappearance of a peak in the spectrum.
- The intensity of infrared absorbances obeys the Beer-Lambert law and may be used for quantitative analysis.

6.0 Tutor-Marked Assignments

1. Discuss the use of IR in structure elucidation
2. Descripe how IR spectroscopy can be used to monitor a reaction.
3. What do you understand by the term ‘fingerprinting’?

7.0 References and Other Sources

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 242-247.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 114-134.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 166-169.

MODULE 2 MASS SPECTROMETRY

UNIT 1 PRINCIPLES OF MASS SPECTROMETRY

1.0 Introduction

Mass spectrometry uses an instrument known as a mass spectrometer and as the name suggests, it is mainly used to determine relative molecular masses. It is also used to investigate the structures of molecules.

2.0 Objectives

From this unit, you will:

- understand the principles of mass spectrometry
- know the different components of a mass spectrometer
- know what a molecular ion is.
- be able to Identify a mass spectrum

3.0 Main Content

3.1 Principles of Mass Spectrometry

Mass Spectrometry (MS) is an analytical technique whereby materials are ionized and dissociated into fragments characteristic of the molecule(s) or elements present in the sample, which is used to obtain information for qualitative and quantitative analysis. Mass spectrometry uses an instrument called the mass spectrometer. The ions formed from the molecules or atoms of a sample are separated in space and detected according to their mass-to-charge ratio, m/z. The numbers of ions of each mass detected constitutes a mass spectrum, which, which may be represented graphically or tabulated. Peak intensities are expressed as a percentage of that of the most abundant ion which is designated the base peak. The spectrum provides structural information and often an accurate relative molecular mass from which an unknown compound can be identified or structure confirmed. Quantitative analysis is based on measuring the numbers of a particular ion present under closely controlled conditions.

3.2 The Mass Spectrometer

There are several types of mass spectrometers available, but they all operate under the same principle. Mass spectrometers have FOUR fundamental parts, namely the sample inlet system, the ion source, the mass analyser and the detector (see Fig. 2.1). The spectrometer is operated under high vacuum of 10^{-4} to 10^{-7} Nm^{-2} to give ions a reasonable chance of travelling from one end of the instrument to the other without any hindrance from air molecules. The entire operation of the mass spectrometer, and often the sample introduction process also is under comple data system control on modern mass spectrometers.

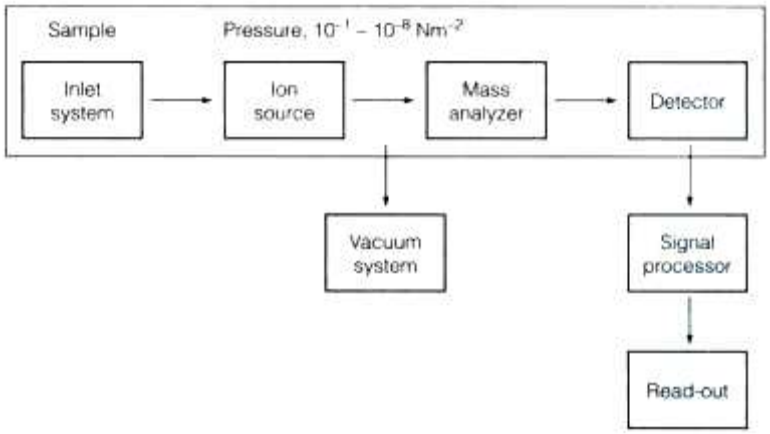


Fig. 2.1. Block diagram of a mass spectrometer

The sample under investigation is introduced into the ionisation source/chamber of the instrument. Once inside the ionisation source the sample molecules are ionised, because ions are easier to manipulate than neutral molecules. Various types of ionization techniques are used in mass spectrometry such as electron impact (EI), chemical (CI), fast atom bombardment (FAB) and electrospray (ESI) ionization techniques. These techniques will be discussed in unit 2 of this module. Ions formed in the ionisation chamber are accelerated along a curved tube and through a strong magnetic field into the analyser region of the mass spectrometer where they are separtated according to their mass (m) –to-charge (z) ratios (m/z). The separated ions are detected and this signal sent to a data system where the m/z ratios are stored together with their relative abundance for presentation in the format of a mass spectrum.

3.3 THE MOLECULAR ION

The molecular ion is often given the symbol M^+ or $M^{\bullet+}$ - the dot in this second version represents the fact that somewhere in the ion there will be a single unpaired electron. It is usually the one half of what was originally a pair of electrons - the other half is the electron which was removed in the ionisation process.

The molecular ions tend to be unstable and some of them break into smaller fragments. In the mass spectrum, the heaviest ion (the one with the greatest m/z value) is likely to be the molecular ion. A few compounds have mass spectra which don't contain a molecular ion peak, because all the molecular ions break into fragments.

3.4 THE MASS SPECTRUM

The most abundant ion in a mass spectrum is arbitrarily given an abundance of 100% and is known as the base peak. The abundance of the other ions is measured relative to the intensity of the base peak. It is not always the heaviest ion in the spectrum. The peaks are typically

very sharp and are often simply represented by vertical lines. The fragmentation patterns of (EI) and (CI) mass spectra are usually significantly different.

The ion formed by the loss of one electron from the molecule in EI spectroscopy is known as the molecular ion. It is usually the most abundant peak on the far right-hand side of the spectrum and its mass is the same as the relative molecular mass (RMM) of the compound. However, it should be noted that some EI spectra of compounds with RMM of less than 300 do not show a molecular ion peak. Furthermore, identification can be complicated by the presence of isotopes in some of the molecular ions collected and counted by the instrument e.g. bromododecane [$CH_3(CH_2)_{11}Br$] has 2 molecular ions with RMM values at 248 and 250 marked as M+. These peaks are due to the presence of bromine isotopes. ^{79}Br and ^{81}Br in the molecules of bromododecane. It should be noted that not all size will depend on the relative abundance of the isotope in the compound (Fig.2.2)

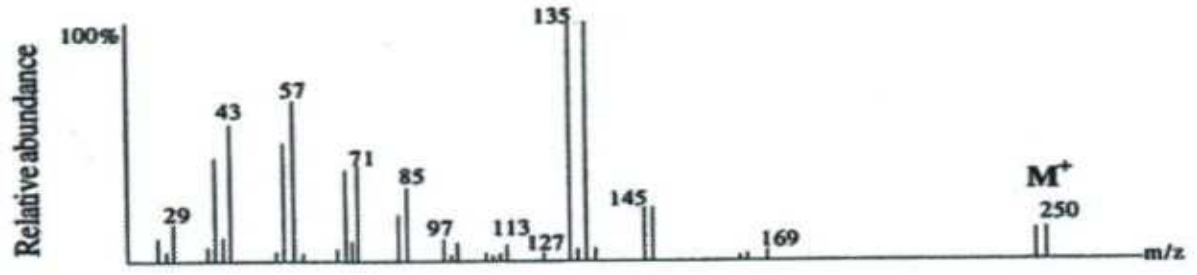


Fig. 2.2 The EI mass spectrum of bromododecane. *Reproduced from G. Thomas. (1996) Chemistry for Pharmacy and the Life sciences. Pearson Education Ltd., Essex, England.*

In CI spectra the peak in the corresponding position to the molecular ion peak of EI spectra is that due to the MH^+ ion whose mass is one unit higher than the RMM of the compound. In rarer circumstances, it may be due to the presence of the $[M-H]^+$ ion whose RMM is one unit less than the RMM of the molecular ion. One of these molecular ion always occurs even in compounds with RMM of less than 300 and so CI spectra are very useful for determining the RMM of these compounds.

4.0 Conclusion

You have now learnt the principles of mass spectrometry and you should now know the different components of a mass spectrometer. Furthermore you have learnt what to expect from a mass spectrum.

5.0 Summary

- Materials are ionized in mass spectrometry and dissociated into fragments characteristic to the molecules present in the sample.
- Mass spectrometry uses an instrument known as the mass spectrum.

- Mass spectrometers consists of four fundamental parts namely- the sample inlet, ion source, mass analyzer and the detector.
- The mass spectrum is a plot of relative abundance versus mass-to-charge ratio,
- The most abundant ion in a mass spectrum is known as the base peak.
- The heaviest ion in the mass spectrum is likely to be the molecular ion.

6.0 Tutor-Marked Assignments

1. Describe the principles of mass spectrometry
2. Describe the features of a mass spectrometer.
3. Mention differences you may expect between an EI and CI mass spectra.
4. What is a molecular ion?.
5. What is the base peak?

7.0 References and Other Sources

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed)*. 270-282.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 178-180.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 166-169

UNIT 2: SAMPLE INTRODUCTION, IONIZATION TECHNIQUES AND ANALYZERS USE IN MASS SPECTROMETRY

1.0 Introduction

In this unit, the different parts of the mass spectrometer will be looked at individually to understand the various features of the different components and the advantages of one feature over another.

2.0 Objectives

In this unit, you will be able:

- to understand the different techniques used in introducing samples into a mass spectrometer.
- to know the various ionization techniques used in MS and understand their features.
- to know the various mass analyzers used in MS and understand the differences.
- to know about the various detectors used in mass spectroscopy.

3.0 Main Content

3.1 Sample Introduction

The method of sample introduction to the ionisation source often depends on the ionisation method being used as well as the type of complexity of the sample. The sample can be inserted directly into the ion source or can undergo some type of chromatography *en route* to the ionisation source. This latter method of sample introduction usually involves the mass spectrometer being coupled directly to a high pressure liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE) separation column, and hence the sample is separated into a series of components which then enter the mass spectrometer sequentially for individual analysis.

3.2 Methods of Sample Ionisation

Many ionisation methods are available and each has its own advantages and disadvantages. The ionisation method to be used depends on the type of sample under investigation and the mass spectrometer available.

a. Electron Impact Ionisation (EI)

The vapour of the sample is bombarded with a stream of high energy electrons. The energy transferred form these electrons to the molecules of the sample causes the molecules of the sample (M) to form molecular ions initially. These are radical cations. These then decompose into smaller fragments.

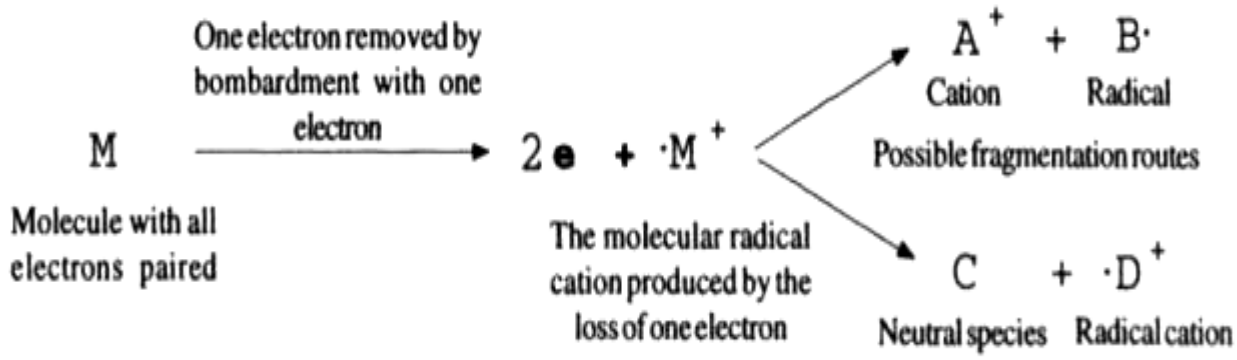


Fig. 2.3 A Schematic representation of the formation of neutral and positively charged ions by EI in a mass spectrometer.

b. Chemical Ionisation (CI)

Chemical ionisation is a softer technique than EI, ions being produced by collisions between sample molecules and ions generated by reagent gas such as methane or ammonia. Three stages are involved for methane for example:

i) reagent gas ionized by EI: $CH_4 + e^- \longrightarrow CH_4^{+\cdot} + 2e^-$

ii) secondary ion formation: $CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^+ + CH_3.$

iii) formation of molecular species: $CH_5^+ + M \longrightarrow MH^+ + CH_4$

compared to EI, there is much less fragmentation, but molecular species, MH^+, which is one mass unit higher than the relative molecular mass (RMM) of the analyte is formed.

c. Fast atom bombardment (FAB)

FAB provides an efficient means to analyze polar, ionic, thermally labile and high molecular weight compounds that are not amenable to normal EI/CI analysis. It has found extreme utility in the analysis of polar biomolecules and natural products. FAB experiments are routinely conducted up to 1000 amu, with higher masses requiring additional effort. In the FAB experiment, a sample that has been dissolved in a suitable matrix is inserted into the mass spectrometer and bombarded with 8-15keV Cs^+ ions. Following ionization, the selected positive or negative ions are extracted, accelerated and then mass analyzed. The FAB mass spectrum is characterized by peaks corresponding to matrix cluster ions, analyted ions, ions representing impurities, and ions of other matrix modifiers (e.g. trifluoroacetic acid) that were added in an attempt to increase the analyted ion abundance.

Successful ionization of FAB is deeply dependent on the matrix selected for the analysis. The successful matrix must meet several requirements. The primary requirement is that the sample MUST be soluble in the matrix. In addition, the matrix must be a low volatility solvent which will not rapidly evaporate in the high vacuum system of the mass spectrometer. Thus, the matrix/sample will maintain its liquid nature in the vacuum system. Several

successful matrices which have been widely used include glycerol, thioglycerol, nitrobenzyl alcohol, 18-crown ether, 2-nitrophenyloctyl ether, sulfolane, diethanolamine, and triethanolamine.

d. Electrospray Ionsiation

Electrospray Ionisation (ESI) is one of the Atmospheric Pressure ionisation (API) techniques and is well-suited to the analysis of polar molecules ranging from less than 100 Da to more than 1,000,000 Da in molecular weight.

During standard electrospray ionisation, the sample is dissolved in a polar, volatile solvent and pumped through a narrow, stainless steel capillary (75 -150 µi.d) at a flow rate of between 1 µl/min and 1 ml/min. A high voltage of 3 or 4kV is applied to the tip of the capillary, which is situated within the ionisation source of the mass spectrometer, and as a consequence of this strong electric field, the sample emerging from the tip is dispersed into an aerosol of highly charged droplets, a process that is aided by a co-axially introduced nebulising gas flowing around the outside of the capillary. This gas, usually nitrogen, helps to direct the spray emerging from the capillary tip towards the mass spectrometer. The charged droplets diminish in size by solvent evaporation, assisted by a warm flow of nitrogen known as the drying gas which passes across the front of the ionisation source. Eventually charged sample ions, free from solvent, are released from the droplets, some of which pass through a sampling cone or orifice into an intermediate vacuum region, and from there through a small aperture into the analyser of the mass spectrometer, which is held under high vacuum. The lens voltages are optimised individually for each sample.

e. Desorption techniques

These are used mainly for solid samples that can be deposited on the tip of a heatable probe that is then inserted into the sample inlet through vacuum locks. Molecules are ionized by the application of a high potential gradient (field desorption, FD) or by focusing a pulsed laser beam onto the surface of the sample. In matrix-assisted laser desorption, MALDI, the sample is mixed with a compound capable of absorbing energy from the laser and which results in desorption of protonated sample molecules. These techniques are very soft, give little fragmentation and are especially useful for compounds with a high RMM.

3.3 Mass analyzers

The main function of the mass analyzer is to separate, or resolve the ions formed in the ionisation source of the mass spectrometer according to their mass-to-charge (m/z) ratios. There are a number of mass analyzers currently available e.g. quadrupoles, time-of-flight, magnetic sectors, Fourier transform and quadrupole ion traps.

These mass analyzers have different features including the m/z range covered, the mass accuracy and achievable resolution. The compatibility of different analysers with different ionisation methods varies. For example, all the analysers listed above can be used in conjunction with electrospray ionisation, whereas MALDI is not usually coupled to a quadrupole analyser.

a. Magnetic Sector

In a magnetic sector instrument the ions generated are pushed out of the source by a repeller potential of same charge as the ion itself. They are then accelerated in an electric field of ca 3-8kV and travel through an electrostatic field region so that they are forced to fall into a narrow range of kinetic energies prior to entering the field of a circular magnet. They then adopt a flight path through the magnetic field depending on their charge to mass (m/z) ratio; the large ions are deflected less by the magnetic field:

$$m/z = \frac{H2r2}{2V}$$

where H is the magnetic field strength, r is the radius of the circular path in which the ion travels and V is the accelerating voltage.

At a particular value for H and V, only ions of a particular mass adopt a flight path that enables them to pass through the collector slit and be detected. If the magnetic field strength is varied, ions across a wide mass range can be detected by the analyser; a typical sweep time for the magnetic field across a mass range of 1000 is 5-10 s but faster speeds are required if high-resolution chromatography is being used in conjuction with mass spectrometry. The accelerating voltage can also be varied while the magnetic field is held constant, in order to produce separation of ions on the basis of their kinetic energies.

b. Quadrupole mass analyzer

This consists of a set of four parallel metal rods positioned very closely together, but leaving a small space through the centre. Ions are accelerated into the space between the rods at one end and a DC potential and high frequency RF signal is applied across opposite pairs of rods. This results in ions of one particular m/z value passing straight through the space to a detector at the other end while all others spiral applied to the rods, ions with different m/z ratios can be allowed to reach the detector in turn.

c. Ion trap mass analyzer

The ion trap is a modified version of quadrupole analyzer with a circular polarisable rod and end caps enclosing a central cavity which is able to hold ions in stable circular trajectories before allowing them to pass to the detector in order of increasing m/z value. A particular feature of quadrupole and ion trap analyzers is their ability to scan through a wide range of masses very rapidly, making them ideal for monitoring chromatographic peaks.

e. Tandem mass analyzers

These incorporate several mass analyzers in series. The analyzers do not necessarily have to be the same type, in which case the instrument is a hybrid one. More popular tandem mass spectrometers include those of the quadrupole-quadrupole, magnetic sector-quadrupole, and more recently, the the quadrupole-time-of-flight geometries. This enables ions selected from the first analyzer to undergo collision induced dissociation (CID) with inert gas molecules contained in a collision cell producing new ions which can then be separated by the next analyzer. The technique, known as tandem mass spectrometry, MS-MS is used in the study of decomposition pathways, especially for molecular ions produced by soft ionization techniques. Collision-induced reactions with reactive gases and various scan modes are also employed in these investigations.

Other mass analyzers include Orbitrap, FT-ICR and Time-of Flight analyzers

When charged particles move in electric and magnetic fields the following two laws apply:

$$\mathbf{F} = Q(\mathbf{E} + \mathbf{v} \times \mathbf{B}),$$ (Lorentz force law)

$$\mathbf{F} = m\mathbf{a} = m\frac{\mathrm{d}\mathbf{v}}{\mathrm{d}t}$$ (Newton's second law of motion)

where **F** is the force applied to the ion, *m* is the mass of the particle, **a** is the acceleration, *Q* is the electric charge, **E** is the electric field, and **v** x **B** is the cross product of the ion's velocity and the magnetic field.

This differential equation is the classic equation of motion for charged particles. Together with the particle's initial conditions, it completely determines the particle's motion in space and time in terms of *m*/*Q*. Thus mass spectrometers could be thought of as "mass-to-charge spectrometers". When presenting data in a mass spectrum, it is common to use the dimensionless *m/z*, which denotes the dimensionless quantity formed by dividing the mass number of the ion by its charge number.

Combining the two previous equation yields:

$$\left(\frac{m}{Q}\right)\mathbf{a} = \mathbf{E} + \mathbf{v} \times \mathbf{B}$$

.

This differential equation is the classic equation of motion of a charged particle in vacuum. Together with the particle's initial conditions it determines the particle's motion in space and time. It immediately reveals that two particles with the same *m*/*Q* ratio behave in the same way. This is why the mass-to-charge ratio is an important physical quantity in those scientific fields where charged particles interact with magnetic or electric fields.

The IUPAC recommended symbol for mass is *m.* The IUPAC recommended symbol for charge is *Q*; however, *q* is also very common. Charge is a scalar property, meaning that it can be either positive (+ symbol) or negative (- symbol). Sometimes, however, the sign of the charge is indicated indirectly. Coulomb is the SI unit of charge; however, other units are not uncommon.

The SI unit of the physical quantity *m* / *Q* is kilograms per coulomb.

[*m* / *Q*] = kg/C

The units and notation above are used when dealing with the physics of mass spectrometry; however, the unit less *m/z* notation is used for the independent variable in a mass spectrum. This notation eases data interpretation since it is numerically more related to the unified atomic mass unit of the analyte. The m in *m/z* is representative of molecular or atomic mass and z is representative of the number of elementary charges carried by the ion. Thus an ion of 1000 Da carrying two charges will be observed at *m/z* 500. These notations are closely related through the unified atomic mass unit and the elementary charge.

Although it is rarely done the numerical conversion factor from SI units (kg/C) to *m/z* notation is:

$$(1000\ g/kg) \times e \times N_A$$

where

$$N_A = 6.022 \times 10^{23}\,\mathrm{mol}^{-1}$$
$$e = 1.602 \times 10^{-19}\,\mathrm{C}.$$

3.4 Detection and Recording of Sample Ions

The detector monitors the ion current, amplifies it and the signal is then transmitted to the data system where it is recorded in the form of a mass spectra. The m/z values of the ions are plotted against their intensities to show the number of components in the sample, the molecular weight of each component, and the relative abundance of the various components in the sample.

The type of detector is supplied to suit the type of analyzer. The more common ones are the photomultiplier, the electron multiplier and the micro-channel plate detectors.

4.0 Conclusion

You should now know how best to introduce a particular sample into a mass spectrometer, and how to select the most appropriate ionization technique and mass analyzer to use for particular experiments.

5.0 Summary

- method of sample introduction depends on the ionisation method and the complexity of the sample
- the ionisation method used depend on the type of sample under investigation and the mass spectrometer available.
- The mass analyzer used will depend on the mass-to-charge range to be covered accuracy needed and the achievable resolution.
- Compatibility of different analyzers with different ionisation methods varies e.g. MALDI cannot be coupled to a quadrupole analyzer.
- The detector is supplied to suit the type of analyzer to be used.

6.0 Tutor-Marked Assignments

1. Describe the ways in which a sample can be introduced into the mass spectrum.
2. Discuss two ionization techniques
3. Write on two mass analyzers and their function.

7.0 Further Readings

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 270-282.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 178-180.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 166-169

UNIT 3 INTERPRETATION OF THE MASS SPECTRUM

1.0 Introduction

The production of a molecular ion is often followed by its dissociation or fragmentation into ions and neutral species of lower mass, which in turn may dissociate further. Fragmentation patterns are characteristic of particular molecular structures and can indicate the presence of specific functional groups, thus providing useful information on the structure and identity of the original molecule. The points of cleavage in a molecule are determined by individual bond strengths throughout the structure and additionally, molecular rearrangements and recombinations can occur. Fragmentation patterns are an invaluable aid in the interpretation of mass spectra and in the identification or confirmation of structural features.

2.0 Objectives

You will learn:

- The general rules used in the interpretation of mass spectrum
- How to identify fragments that are peculiar to a particular functional group.

3.0 Main Content

3.2 Interpretation

Mass spectra can be difficult to interprete because of the complexity of the fragmentation. Generally, one identified the peak due to the molecular ion (M^+) or MH^+ ion and uses this as the reference point. The mass differences between this peak and other peaks are determined and the likely nature of the fragments lost from the reference peak deduced from reference tables. One can work the other way round and consult tables which indicate the most likely composition of a fragment. The peaks on either side of the main peak may be formed because of the presence of isotopes in a fragment. These peaks must be taken into account when making any deductions, and initially it is best to consider only the most abundant peaks. Unfortunately, the great diversity of fragments produced in a mass spectrum means that these deductions are of limited value on their own but taken in conjunction with other experimental evidence can be of considerable use either in identifying or determining the structure of a compound.

3.2.1 SOME RULES USED IN THE INTERPRETATION OF MASS SPECTRA

1. The nitrogen rule states that compounds with an even numbered RMM must contain zero or an even number of nitrogen atoms, and those with an odd-numbered RMM must contain an odd number of nitrogen atoms.

2. The unsaturated sites rule provides a means of calculation of the no of double-bond equivalent in a molecule from the formula:

No of C atoms + 1/2(no of N atoms) – ½(no of H + halogen atoms) + 1

For example, or C_7H_7ON, the formula gives 7+0.5-3.5-1 = 5 double bond equivalents. This corresponds to benzamide, $C_6H_5CONH_2$, the aromatic ring being counted as three double bonds plus one for the ring.

3. The intensity of the molecular ion peak decreases with increasing chain in length in the spectra of a homologous series of compounds and with increased branching of the chain.

4. Double bonds are cyclic structures tend to stabilize the molecular ion, saturated rings losing side chains at the α-position.

5. Alkyl-substituted aromatic rings (benzyl group) undergo rearrangement to form a tropylium cation $C_7H_7^+$ (see below) giving a prominent peat at m/z 91.

6. Small neutral molecules such as CO, C_2H_4, C_2H_2, H_2O and NH_3 are often lost during fragmentation.

7. The C-C bond adjacent to a heteroatom (N,O,S) is frequently cleaved leaving the charge on the fragment containing the heteroatom (Y), whose nonbonding electrons provide resonance stabilization e.g

$$CH_3\text{-}CH_2\text{-}Y^{+\bullet}\text{-}R \xrightarrow{-CH_3^{\bullet}} CH_2{=}Y^{+}\text{-}R \longleftrightarrow {}^{\bullet}CH_2\text{-}Y\text{-}R$$

8. McLafferty rearrangements

McLafferty rearrangement occur in carbonyl compounds e.g

$$HCH_2\text{-}CH_2\text{-}CH_2\text{-}CO^{+\bullet}\text{-}OR \longleftrightarrow C_2H_2 + CH_2{=}C(O^{+\bullet})\text{-}OR$$

A neutral molecule of ethene is lost in the process.

The mass spectrum of toluene (methyl benzene) is shown below (Fig.2.4). The spectrum displays a strong molecular ion at m/z = 92, small m + 1 and m +2 peaks, a base peak at m/z = 91 and an assortment of minor peaks m/z =65 and below.

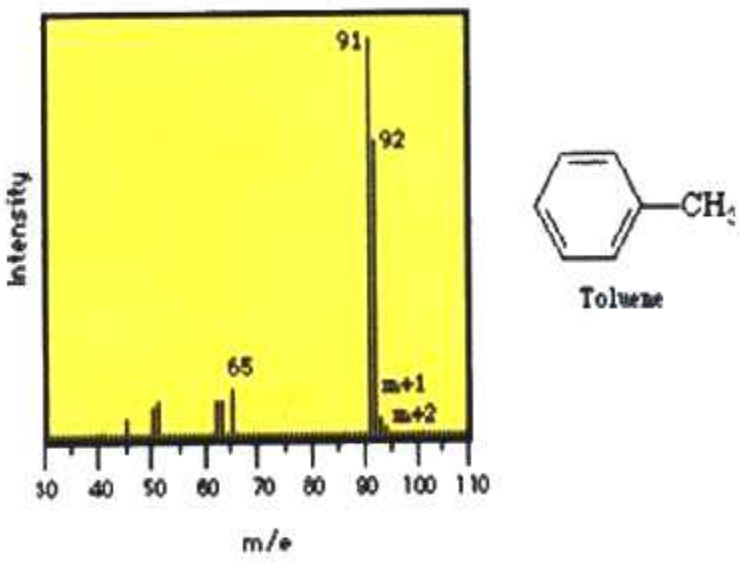
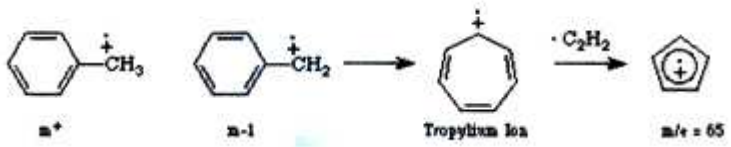


Figure 2.4: Mass Spectrum of toluene

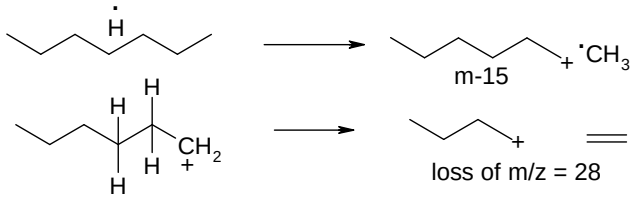
The molecular ion, again represents loss of an electron and the peaks above the molecular ion are due to isotopic abundance. The base peak in toluene is due to loss of a hydrogen atom to form the relatively stable benzyl cation. This is thought to undergo rearrangement to form the very stable tropylium cation, and this strong peak at m/z = 91 is a hallmark of compounds containing a benzyl unit. The minor peak at m/z =65 represents loss of a neutral acetylene from the tropylium ion and the minor peak below this arise from more complex fragmentation.



3.2.2 FRAGMENTATION PATTERN FOR DIFFERENT FUNCTIONAL GROUPS

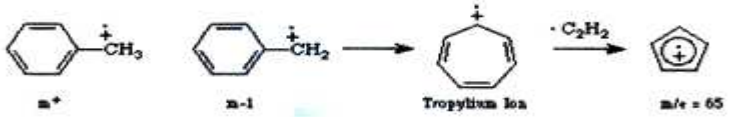
a. Alkanes

Simple alkanes tend to undergo fragmentation by the initial loss of a methyl group to form a (m-15) species. This carbocation can then undergo stepwise cleavage down the alkyl chain, expelling neutral two-carbon units (ethane). Branched hydrocarbons form more stable secondary and tertiary carbocations, and these peaks will tend to dominate the mass spectrum.



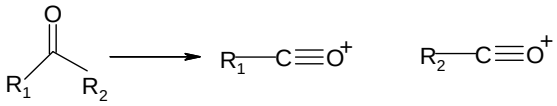
b. Aromatic Hydrocarbons

The fragmentation of the aromatic nucleus is somewhat complex, generating series of peaks having m/z = 77, 65,63 etc. While these peaks are difficult to describe in simple terms, they do form a pattern (the "aromatic cluster") that becomes recognizable with experience. If the molecule contains a benzyl unit, the major cleavage will be to generate the benzyl carbocation, which rearranges to form the tropylium ion. Expulsion of acetylene (ethyne) from this generates a characteristic m/z = 65 peak.

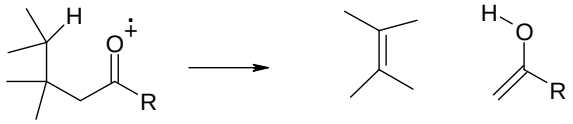


c. Aldehydes and Ketones

The predominate cleavage in aldehydes and ketones is loss of one of the side chains to generate the substituted oxonium ion. This is an extremely favourable cleavage and this ion often represents the base peak in the spectrum. The methyl derivative ($CH_3C{\equiv}O^+$) is commonly referred to as the "acylium ion".

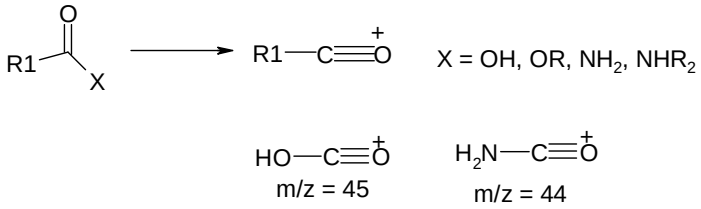


Another common fragmentation observed in carbonyl compounds (and in nitriles, etc) involves the expulsion of neutral ethane via a process known as the McLafferty rearrangement, following the general mechanism shown below.



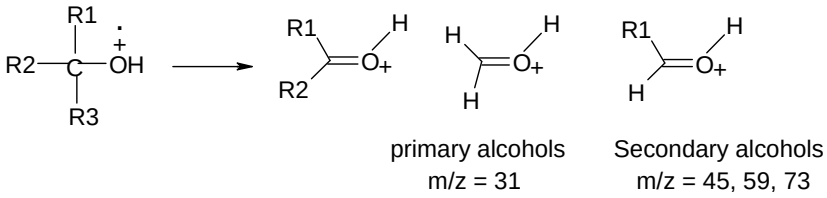
d. Esters, acids and amides

As with aldehydes and ketones, the major cleavage observed for these compounds involves expulsion of the "X" group, as shown below, to form the substituted oxonium ion. For carboxylic acids and unsubstituted amides, characteristic peaks at m/z = 45 and 44 are also often observed.



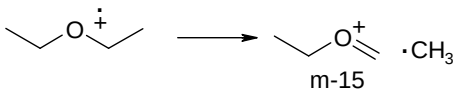
e. Alcohols

In addition to losing a proton and hydroxyl radical, alcohols tend to loose on of the α-alkyl groups (or hydrogens) to form the oxonium ions shown below. For primary alcohols, this generates a peak at m/z = 31; secondary alcohols generated peaks with m/z = 45, 59,73 etc., according to substitution.



f. Ethers

Following the trend of alcohols, ethers will fragment often by loss of an alkyl radical, to form a substituted oxonium ion, as shown below for diethyl ether.



g. Halides

Organic halides fragment with simple expulsion of the halogen, as shown below. The molecular ions of chlorine and bromine-containing compounds will show multiple peaks due to the fact that each of these exists as two isotopes in relatively high abundance. Thus for chlorine, the $^{35}Cl/^{37}Cl$ ratio is roughly 3.08:1 and for bromine, the $^{79}Br/^{81}BrBr$ ratio is 1.02:1. The molecular ion of a chlorine-containing compound will have two peaks, separated by two mass units, in the ration ~ 3:1, and a bromine-containing compound will have two peaks, again separated by two mass units, having approximately equal intensities.

The lists given above are by no means exhaustive and represents only the simplest and most common fragments seen in the mass spectrum.

3.3 Examples of mass spectral interpretation

a. Figure 2.5 below are the mass spectral data of octane and 2,2,4-trimethylpentane. Octane is a saturated straight chain so the spectra is characterized by clusters of peaks 14

mass units (CH2 groups) apart, as successive C-C bonds along the chain are cleaved in different molecules. Octane has a base pear at m/z 43 due to the $CH_3CH_2CH_2^+$ fragment ion, and

Table 2.1 Commonly Lost fragments from a Molecular ion

Loss amu	Radicals/neutral fragment lost	Interpretation
1	$H^{\bullet}$	Often a major ion in amines, alcohols and aldehydes
2	H_2	
15	$CH_3^{\bullet}$	Most readily lost from a quaternary carbon
17	$OH^{\bullet}$ or NH_3	
18	H_2O	Readily lost from secondary or tertiary alcohols
19/20	$F^{\bullet}$/HF	Fluorides
28	CO	Ketones or acid
29	C_2H_5	
30	CH_2O	Aromatic methyl ester
31	$CH_3O^{\bullet}$	Methyl ester/methoxime
31	CH_3NH_2	Secondary amine
32	CH_3OH	Methyl ester
33	$H_2O + CH_3^{\bullet}$	
35/36	$Cl^{\bullet}$/HCl	Chloride
42	$CH_2=C=O$	Acetate
43	$C_3H_7^{\bullet}$	Readily lost if isopropyl group present
43	$CH_3CO^{\bullet}$	Methyl ketone
43	$CO + CH_3$	
44	CO_2	Ester
45	$CO_2H^{\bullet}$	Carboxylic acid
46	C_2H_5OH	Ethyl ester
59	CH_3CONH_2	Acetamide
60	CH_3COOH	Acetate
73	$(CH_3)_3Si$	Trimethylsilyl ester
90	$(CH_3)_3SiOH$	Trimethylsilyl ether

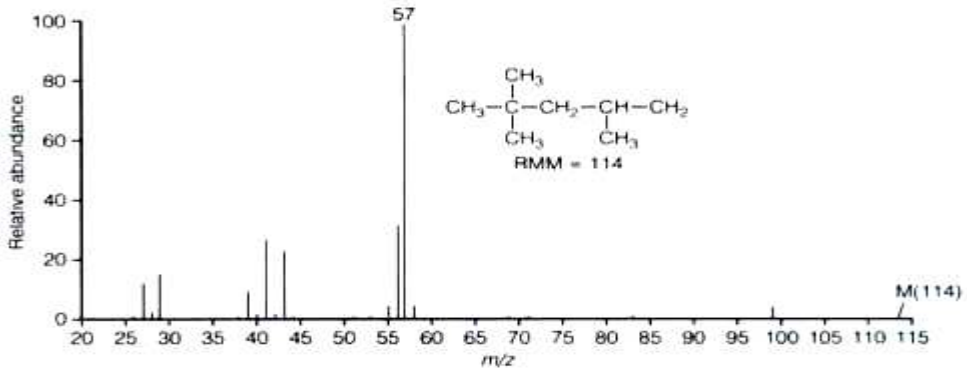


Fig. 2.5 Mass spectrum of 2.2.4-trmethylpentane. *Reproduced from D. Kealey et al. (2002) Analytical Chemistry, BIOS Scientific Publishers Limited, Oxford, UK.*

a small molecular ion peak at m/z (rule 3). Branching of the chain alter the relative intensities of the clusters, as shown by the spectrum of the isomeric 2,2,4-trimethylpentane, which has a base peak at m/z 57 due to the $(CH_3)_3C^+$ fragment ion, and no significant m/z 71, 85 or molecular ion peak (rule 3).

b. The spectrum of methylbenzene (Fig 2.6) typifies alkyl-substituted aromatic compounds, with a base peak corresponding to the tropylium ion, $C_7H_7^+$, at m/z 91 and a large molecular ion peak at m/z 92 (rules 4 and 5).

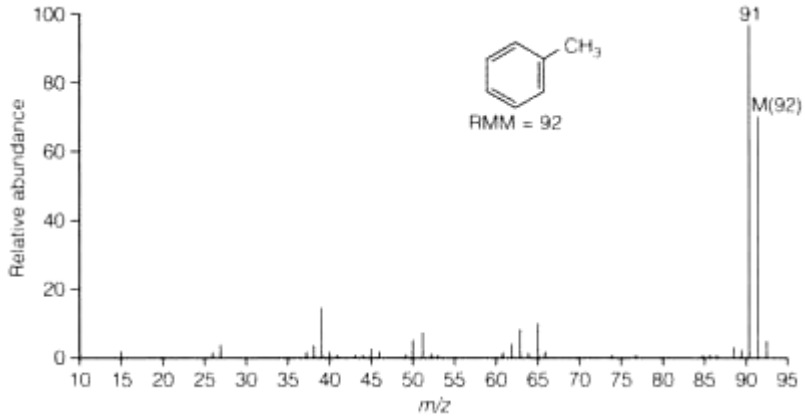


Fig. 2.6 Mass spectrum of methylbenzene. *Reproduced from D. Kealey et al.(2002) Analytical Chemistry, BIOS Scientific Publishers Limited, Oxford, UK.*

4.0 Conclusion

You should now be able to interpret a mass spectrum based on the rules discussed in this unit and your knowledge on the fragmentation pattern expected from different functional groups.

5.0 Summary

- Fragmentation patterns are characteristic of a particular molecule and indicate the presence of specific functional groups.

- Fragmentation patterns are invaluable in the interpretation and identification of structural features.
- Even no numbered RMM contain zero or even numbered nitrogen.
- Carbonyl compounds undergo McLafferty rearrangements resulting in loss of ethene.
- Halides show multiple peaks due to the existence of two isotopes in relatively high abundance.

6.0 Tutor-Marked Assignments

1. Discuss some of the rules that may be used in the interpretation of a mass spectrum.
2. Which fragmentation pattern would you expect from:
    a) An alkane
    b) A ketone
    c) Halides.
    d) An alcohol

3. Identify the fragments corresponding to the peaks in the mass spectrum below (Figure 2.7):

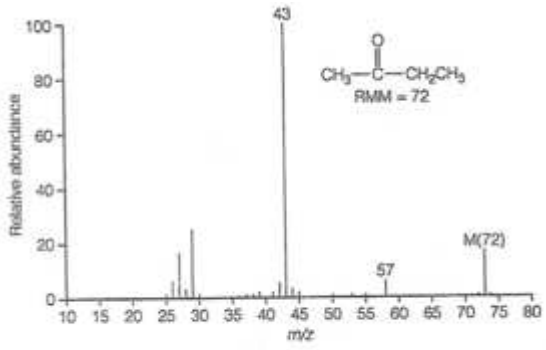


Figure 2.7: The mass spectrum of butanone. *Reproduced from D. Kealey et al. (2002) Analytical Chemistry, BIOS Scientific Publishers Limited, Oxford, UK.*

7.0 References

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 270-282.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 178-180.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 166-169

UNIT 4: APPLICATIONS OF MASS SPECTROMETRY

1.0 Introduction

The main use of mass spectrometry is for RMM and structure determination (discussed in the unit 3). It is always used in conjunction with information from other sources. Other applications include use in conjunction with gas chromatography (GC) and HPLC to provide structural information about the component of mixtures as they elute from the column. They are also used to a lesser extent as detectors in gas chromatography.

2.0 Objectives

- To be able to appreciate the usefulness of mass spectrometry in other applications.
- To understand how mass spectrometry can be applied to various analytical problems.
- To know how MS can be interfaced with some chromatographic techniques.

3.0 Main Content

3.1 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography (GC) was the earliest chromatographic technique to be interfaced with mass spectrometer. The original type of gas chromatograph had a packed GC column with a gas flow rate passing through it at ca. 20ml/min and the major problem was how to interface the GC without losing the mass spectrometer vacuum. This was resolved by the use of a jet separator, where the column effluent was passed across a very narrow gap between two jets and the highly diffusible carrier gas was largely removed, whereas the heavier analyte molecules crossed the gap without being vented. The problem of removing the carrier gas no longer exists since GC capillary columns provide a flow rate of 0.5-2 ml/min, which can be directly introduced into the mass spectrometer without losing vacuum.

3.1.1 Ionisation techniques used in GC-MS

There are three main types of ionisation techniques used in GC-MS.

a) Electron impact

This method has already been discussed in unit 2 of this module.

b) Positive ion chemical ionisation (PICI)

CI has already been discussed under unit 2. However in this case the positively charged ions can either associate with the analyte or transfer a proton to the analyte.

c) The most common form of ionisation occurring in the case of negative ion spectra is electron capture ionisation. A reagent gas is used to collide with it so that their energies are reduced to <10eV. Molecules with a high affinity for electrons are able to capture these low-energy thermal electrons. This is often loosely called NICI but since it does not involve formation of a chemical adduct, it is strictly chemical ionisation. The two commonly observed types of electron capture are shown below:

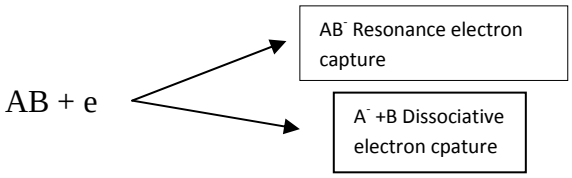


Figure 2.8: Electron capture in NICI

3.1.2 Application of GC-MS in impurity profiling

GC-MS has found a role in impurity identification in the pharmaceutical industry. Such impurities can arise either from the manufacturing process or from degradation of the drug.

3.2 Liquid Chromatography-mass spectrometry (LC-MS)

The interfacing of a liquid chromatograph to a mass spectrometer proved much more difficult than interfacing a gas chromatograph since each mole of solvent introduced into the instrument produces 22.4l of solvent vapour even at atmospheric pressure. The technique has made huge advances in the last 10 years and there are many types of interface available, the most successful of which are the electrospray and atmospheric pressure ionisation sources. Below are a list of some of the interfaces used in LC-MS.

a) Thermospray

The eluent from the column is vapourised and a portion of the vapour (ca 1%) is transferred to the mass spectrometer and the rest of the vapour is pumped to waste. The spectra produced are like CI spectra since the presence of the solvent vapour with the sample reduces the energy of the ionisation process and adducts can be formed with the solvent. Sensitive to the 10^{-9} g level; mass range up to 2000 amu.

b) Electrospray (ES) Ionisation

This is the most common LC-MS interface. Flow rates up to 1 ml/min but best at 200 µl/min or below. A charged aerosol is generated at atmospheric pressure and the solvent is largely stripped away with a flow of N_2 gas. The charged molecules are drawn into the MS by electrostatically charged plates. It can be used to determine both small molecules and molecules up to 200000 amu. Spectra can be simple, containing molecular ion only, or fragmentation can be induced by varying the cone voltage. ES ionisation is more suitable for polar molecules. The advantage of ESI is that large molecules which are not volatile enough to evaporate by heating can be introduced into the gas phase. The aminoglycoside antibiotic kanamycin is an example of an extremely polar compound for which ESI is the ideal ionisation technique.

c) Atmospheric pressure ionisation

This method is very similar to ES, but can operate at normal LC flow rates of 0.2-2ml/min. ES instruments can be simply converted to run this technique. Ionisation is more analogous to CI, with the corona discharge producing ions such as H_3O^+ and $N2^+$, which promote the ionisation of the sample. This method is complementary to ES since this interface will ionise less polar molecules.

d) Matrix-assisted laser desorption with time of flight (MALDI-TOF)

This can be used for very large protein > 200 000MU. The sample is dissolved in a light-absorbing matrix; soft ionisation is promoted by a pulsed laser; and ions are ejected from the matrix and accelerated using an electrostatic field into a field-free region. The lighter ions travel fastest. In order to improve resolution, a device called a 'reflectron' is used to focus the kinetic energies of a population of a particular ion prior to its entering a field-free region. The length of time taken for ions to reach the detector gives their molecular weight (MW). The pulsed nature of the ionisation ensures there is no overlap between spectra. Ideal technique for characterisation of the MW of large proteins.

e) Ion-trap

The ion trap separates ion by capturing them within a circular electrode, where they orbit until they are ejected by a variation in voltage. The technology is developing rapidly and has advantages over a quadrupole in that ions can be trapped while tandem MS-type fragmentation is produced. It can filter out background while the ion of interest is retained in the trap before being further fragmented and ejected.

f) Tandem mass spectrometry

Since a soft ionisation technique such as ESI produces very little diagnostic fragmentation, it is often used in conjunction with tandem mass spectrometry. The type of mass spectra obtained by using collision induced dissociation (CID) in a tandem mass spectrometer are similar to those which are obtained under EI conditions. Typically, the molecular ion of the molecule is selected (the precursor ion) by the first quadrupole. The selected ion is then fragmented using a second quadrupole, into which argon gas is introduced, which acts as a collision cell. The fragments produced (product ions) are separated using a third quadrupole. The technique can sometimes be used without chromatographic separation, making it a very rapid technique in areas such as clinical screening for diagnostic marker compounds.

3.2. Use of LC-MS in drug metabolic studies

The body metabolises foreign compounds (xenobiotics) such as drugs to make them more polar and water soluble to facilitate excretion from the body. LC-MS can be used to identify these metabolites.

3.3 Drug discovery

Drug discovery involves a number of phases, including target identification, lead identification, small molecule optimization and pre-clinical and clinical development.

Target identification has been speeded up as a result of genomics but the measurement of gene transcription through detection of RNAs does not necessarily indicate exactly what the structures of the proteins produced are, since the proteins may be modified after translation by processes such as glycosylation or phosphorylation. Advances in mass spectrometry have allowed identification of translated proteins. Such proteins may signal disease processes, in which case their regulation by a potential drug might indicate its efficacy, not equally expression of certain proteins following drug therapy may indicate drug toxicity.

4.0 Conclusion

You have now learnt applications of MS when combined with chromatographic techniques such as GC and LC. This combination is a very powerful tool in analysis of mixtures and it is time saving.

5.0 Summary

- MS can be interfaced with GC
- MS can be interfaced with LC
- GC-MS and LC-MS are used for impurity profiling in pharmaceutical industries.
- Interfacing GC with MS requires the use of a jet separator.
- The three main type of ionisation techniques used in MS is EI, PICI and NICI.
- The most common LC-MS interface is ESI, APCI compliments ESI as used for non-polar compounds.

6.0 Tutor-Marked Assignment

1. Write on two applications of MS
2. Discuss how GC-MS can be used in impurity profiling
3. How can GC be interfaced with MS without losing the MS vacuum
4. Mention two interfaces used in LC-MS

7.0 References and Other sources.

Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 201-213.

MODULE 3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Unit 1 Principles of Nuclear Magnetic Spectroscopy

Unit 2 Proton (^{1}H)- NMR

Unit 3 Carbon (^{13}C)- NMR

Unit 4 Two dimensional NMR and other applications of NMR

Unit 5 Structure elucidation of organic molecules with worked example

UNIT 1 PRINCIPLES OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

1.0 Introduction
2.0 Objectives
3.0 Main Content
    3.1 Principles of NMR spectroscopy
    3.2 Chemical Shift
    3.3 NMR spectrometers
4.0 Conclusion
5.0 Summary
6.0 Tutor-Marked Assignment
7.0 Reference/Further Reading

1.0 Introduction

Nuclear Magnetic resonance spectroscopy (NMR) is concerned with the absorption of electromagnetic radiation in the radiofrequency range by the nuclei of some isotopes. These isotopes behave as though they are spinning charged particles and generate a magnetic field along the axis about which they are spinning. As a result, these nuclei can be pictured as tiny spinning bar magnets which in the absence of a strong external magnetic field, are randomnly orientated in space. When a strong external magnetic field is applied to these spinning magnets, the magnetic fields interact and the spinning magnets assume different orientations in the external field. Each relative direction of alignment is associated with an energy level. Only certain well-defined energy levels are permitted; that is, the energy levels are quantized. Hence the nucleus can become aligned only in well-defined directions relative to the magnetic field B_0. (Note: The symbol B is the SI symbol for magnetic field; many texts still use the symbols H and H_0 for magnetic field).

The number of orientations or number of magnetic quantum states is a function of the physical properties of the nuclei and is numerically equal to 2I +1. Where I is the spin quantum number. It is useful to consider three types of nuclei.

Type 1: Nuclei with I = 0. These nuclei do not interact with the applied magnetic field and are not NMR chromophores. Nuclei with I = 0 have an even number of protons and even number of neutrons and have no net spin. This means that nuclear spin is a property characteristic of certain isotopes rather than certain elements. The most prominent examples of nuclei with I = 0 are ^{12}C and ^{16}O, the dominant isotopes of carbon and oxygen. Both carbon and oxygen have isotopes that can be observed by NMR spectroscopy.

Type 2: Nuclei with I =½. These nuclei have a non-zero magnetic moment and are NMR visible and have no nuclear electric quadrupole (Q). The two most important nuclei for NMR spectroscopy belong to this category: ^{1}H (ordinary hydrogen) and ^{13}C (a non-radioactive isotope of carbon occurring to the extent of 1.06% at natural abundance). Also, two other commonly observed nuclei ^{19}F and ^{31}P have I = ½. Together, NMR data for ^{1}H and ^{13}C account for well over 90% of all NMR observations in the literature and the discussion and examples in this module all refer to these two nuclei. However, the spectra of all nuclei with I = ½ can be understood easily on the basis of common theory.

Type 3: Nuclei with I > ½. These nuclei have both a magnetic moment and an electric quadrupole. This group includes some common isotopes 9 e.g. ^{2}H and ^{14}N) but they are more difficult to observe and spectra are generally very broad. This group of nuclei will not be discussed further.

Certain nuclei notably ^{1}H and ^{13}C, can only take up two orientations in this field, a low energy parallel orientation in which the magnetic field of the nucleus is aligned in the same direction as that of the external field and an antiparallel high energy orientation where the nuclear magnetic field is opposite to that of the external field. Under normal conditions there

are slightly more nuclei in the lower energy parallel orientation than the higher energy antiparallel orientation. A number of isotopes such as ^{14}N, ^{19}F and ^{31}P also exhibit nuclear magnetic resonance in a strong magnetic field.

In the case of 1H, where I = ½, the number of orientations is 2 x (1/2)+1 = 2. Consequently, for 1H only two energy levels are permitted, one with m = ½ and the other with m = -1/2. The splitting of these energy levels in a magnetic field is called nuclear Zeeman splitting. When a nucleus with I = ½, such as 1H, is placed in an external magnetic field, its magnetic moment lines up in one of two directions, with the applied field or against the applied field. This results in two discrete energy levels, one of higher energy than the other, as shown in Fig. 3.1. The lower energy level is that where the magnetic moment is aligned with the field. The lower energy state is energetically more favoured than the higher energy state, so the population of the nuclei in the lower energy state will be higher than the population of the higher energy state. The difference in energy between levels is proportional to the strength of the external magnetic field. The axis of rotation also rotates in a circular manner about the external magnetic field axis, like a spinning top.

The basis of NMR experiment is to cause a transition between these two states by absorption of radiation. A transition between these two energy states can be brought about by absorption of radiation with a frequency that is equal to $\Delta E = he$. The difference in energy between the two quantum levels of a nucleus with I = ½ depends on the applied magnetic field B_0 and the magnetic moment μ of the nucleus. The relationship between these energy levels and the frequency v of absorbed radiation is as follows:

$$V = \gamma\frac{Bo}{2\pi} \text{ or } \omega = \gamma B_0 \qquad (1.1)$$

Where γ is the magnetogyric ration; B_0, the applied magnetic field; v is frequency of absorbed radiation and ω is the frequency in units of rads/s. Equation (1.1) is the Larmor equation, which is fundamental to NMR. It indicates that for a given nucleus there is a direct relationship between the frequency ω of RF radiation absorbed by that nucleus and the applied magnetic field B_0. This relationship is the basis of NMR.

Objectives

You will learn

- the principles of Nuclear magnetic resonance spectroscopy
- the meaning of chemical shift
- about the different components of a NMR spectrometer and how it works.

3.0 Main Content

3.1 Principles of Nuclear Magnetic Resonance Spectroscopy

Absorption of electromagnetic radiation in the radiofrequency region by ^{1}H, ^{13}C and other suitable nuclei when placed in a strong magnetic field can cause the nuclei in the lower energy parallel state to spin-flip to the higher energy antiparallel state. When this occurs the nucleus is said to be in resonance and the absorbed radiation is commonly referred to as a signal. Nuclei do not stay in the higher energy state but dissipate their energy through so-called relaxation processes, the exact nature of which is not understood. If relaxation did not occur, all the nuclei in a sample would eventually be promoted to the higher energy state there would be non left to absorb the radiofrequency energy. In other words, no absorption signal would be observed. However this does not happen unless the sample is irradiated with radiation of such high intensity that all the nuclei are forced to remain in the higher energy state. In this situation the sample is said to be saturated. Nuclear magnetic resonance spectrometers have to be adjusted so that the intensity of the radiofrequency radiation being used does not cause this to happen.

Resonance can be brought about in two ways: either the external field can be kept constant and the radiofrequency varied, or the radiofrequency is kept constant and the external magnetic field varied. The former is known as a frequency sweep whilst the latter is known as a field sweep. In practice most instruments use a field sweep as it is easier to achieve a homogenous magnetic field. The amount of energy needed to cause nuclei to resonate depends on the external magnetic field, the isotope and its molecular environment. For example, with an external magnetic field strength of 14,000 gauss, a radiofrequency of the order of 60 megahertz (MHZ) is required to cause protons (^{1}H nuclei) to resonate whilst a radiofrequency of the order of 25.14 MHZ is necessary to bring ^{13}C nuclei into the resonance. Other field strengths can be used, some instruments use field strengths up to 140,000 gauss. These machines are very sensitive but require very high radiofrequencies to cause resonance. For example, a machine with a field strength of 234,900 gauss uses a radiofrequency of the order of 100 MHZ to bring ^{1}H nuclei to resonance.

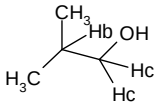
Since all the nuclei of an isotope are identical, one would expect all the ^{1}H nuclei to absorb at the same frequency, Similarly, all the ^{13}C nuclei would be expected to absorb at the same frequency but a different one from the ^{1}H nuclei. However, this is not the case because the actual frequency of the absorption will depend on the electrons in the structure which are also influenced by the external magnetic field (H_o). This field is believed to cause the electrons to circulate the nucleus in a plane perpendicular to H_o. This produces a small magnetic field in the opposite direction to H_o which reduces the effect of the external magnetic field experienced by the nucleus. The nucleus is said to be shielded. As a result, the magnetic field H_E actually experienced by a particular nucleus in the compound will be the resultant of the external magnetic field and the small opposing local fields due to the electron clouds of its neighbouring nuclei. As each nucleus in a molecule is in a slightly different electron environment it will experience a slightly different degree of shielding relative to the other nuclei in the molecule. Therefore, each nucleus will require a slightly different

radiofrequency to cause it to resonate. The resonance difference between the resonance of a nucleus and the resonance of the reference compound is termed the **chemical shift** (see section 3.2 below).

The resonance frequencies of ^{1}H and ^{13}C atoms are measured relative to a reference point, usually tetramethylsilane (TMS). Tetramethylsilane is used as an internal standard for both ^{1}H and ^{13}C spectroscopy because it has a single sharp proton absorption signal that occurs above the signals of most organic molecules. For convenience, the spectra are recorded as plots of signal intensity against chemical shift where the chemical shift of TMS is arbitrarily set at zero.

Each ^{1}H nucleus is shielded or screened by the electrons that surround it. Consequently each nucleus feels the influence of the main magnetic field to a different extent, depending on the efficiency with which it is shielded. Each ^{1}H nucleus with a different chemical environment has a slightly different shielding and hence a different chemical shift in the ^{1}H-NMR spectrum. Conversely, the number of different signals in the ^{1}H-NMR spectrum reflects the number of chemically distinct environments for ^{1}H in the molecule. Unless two ^{1}H environments are precisely identical (by symmetry) their chemical shifts must be different. When two nuclei have identical molecular environments and hence the same chemical shift, they are termed chemically equivalent or isochronous nuclei. Non-equivalent nuclei that fortuitously have chemical shifts that are so close that their signals are indistinguishable are termed accidentally equivalent nuclei. The chemical shift of a nucleus reflects the molecular structure and it can therefore be used to obtain structural information.

Let us consider the proton NMR of 2-methylpropanol below. The 6 alkane methyl protons are in the same chemical environment and far away from the electronegative oxygen hence little affected and thus resonate at a chemical shift of 0.9 ppm. The six protons can be assigned as Ha. The methine (Hb) group is shifted downfield and has a chemical shift of 2.5 ppm. The CH_2 (Hc) protons are shifter further downfield as deshielded by the electronegative oxygen and so resonates at 4.2 ppm, while the OH proton resonates at ~3 ppm.



2-methylpropanol

3.2 Chemical Shift

Chemical shift δ is defined as the ratio of the difference between the frequency of the signal and that of TMS (Hz) and the spectrometer operating frequency (MHz). It has no units and is normally recorded as parts per million (ppm). Chemical shifts in ^{13}C NMR spectroscopy are much larger than those found in ^{1}H NMR spectroscopy.

$$\delta = \frac{\text{Difference between the frequency of the signal and that of TMS (Hz)}}{\text{Spectrometer operating frequency (MHz)}}$$

The chemical shifts of a spectrum recorded on a 60MHz spectrometer will have the same values when the same spectrum is recorded on a 100 MHZ machine even though the absorption occurs at a different radiofrequency for each instrument. Absorption signals that occur to the left of the TMS signal on the spectrum are referred to as being downfield of TMS or deshielded whilst those that occur to the right are referred to as being upfield or shielded. Chemical shift values are not only affected by the nature of the structure but are also influenced by hydrogen bonding, temperature and the solvent.

Any effect which alters the density or spatial distribution of electrons around a ^1H nucleus will alter the degree of shielding and hence it's chemical shift. ^1H chemical shifts are sensitive to both the hybridisation of the atom to which the ^1H nucleus is attached (sp^2, sp^3 etc.) and to electronic effects (the presence of neighbouring electronegative/electropositive groups). The chemical shift of a nucleus may also be affected by the presence in its vicinity of a magnetically anisotropic group (e.g. an aromatic ring or carbonyl group).

Nuclei tend to be deshielded by groups which withdraw electron density. Deshielded nuclei resonate at higher ∂ values (away from TMS). Conversely shielded nuclei resonate at lower ∂ values (towards TMS).

Electron withdrawing substituents (-OH, -OCOR, -OR, $-NO_2$, halogen) attached to an aliphatic carbon chain cause a downfield shift of 2-4 ppm when present at C_α and have less than half of this effect when present at C_β.

3.3 NMR SPECTROMETERS

Spectrometers were originally designed to scan and record an NMR spectrum by progressively changing (sweeping) the applied magnetic field at a fixed radiofrequency (RF), or sweeping the frequency at a fixed field. Sample resonances were recorded as a series of sharp absorption peaks along the frequency/field axis, which is calibrated in ppm. These continuous wave (CW) instruments have been largely superseded by pulsed Fourier transform (FT) spectrometers. Samples are subjected to a series of rapid, high-energy RF pulses of wide frequency range, between which a decaying emission signal from nuclei excited by the pulse and then relaxing to the ground state is monitored by the receiver circuit. The detector signal, or free induction decay (FID), contains all of the spectral information from the sample, but in the form of a time-dependent interferogram. This can be digitized and converted into a conventional spectrum mathematically in less than a second by a computer using a fast Fourier transform (FFT) algorithm. Multiple interferograms can be rapidly accumulated and averaged to increase sensitivity by as much as three orders of magnitude.

A block diagram of a typical NMR spectrometer is shown below and comprises of five main components:

- A superconducting solenoid or electromagnet providing a powerful magnetic field of up to about 17 Tesla;
- A highly stable RF generator and transmitter coil operating at up to about 750MHz;
- A receiver coil with amplifying and recording circuitry to detect and record sample resonances;
- A sample probe positioned between the poles of the magnet;
- A dedicated microcomputer for instrument control, data processing (FFT of interferograms) and data storage.

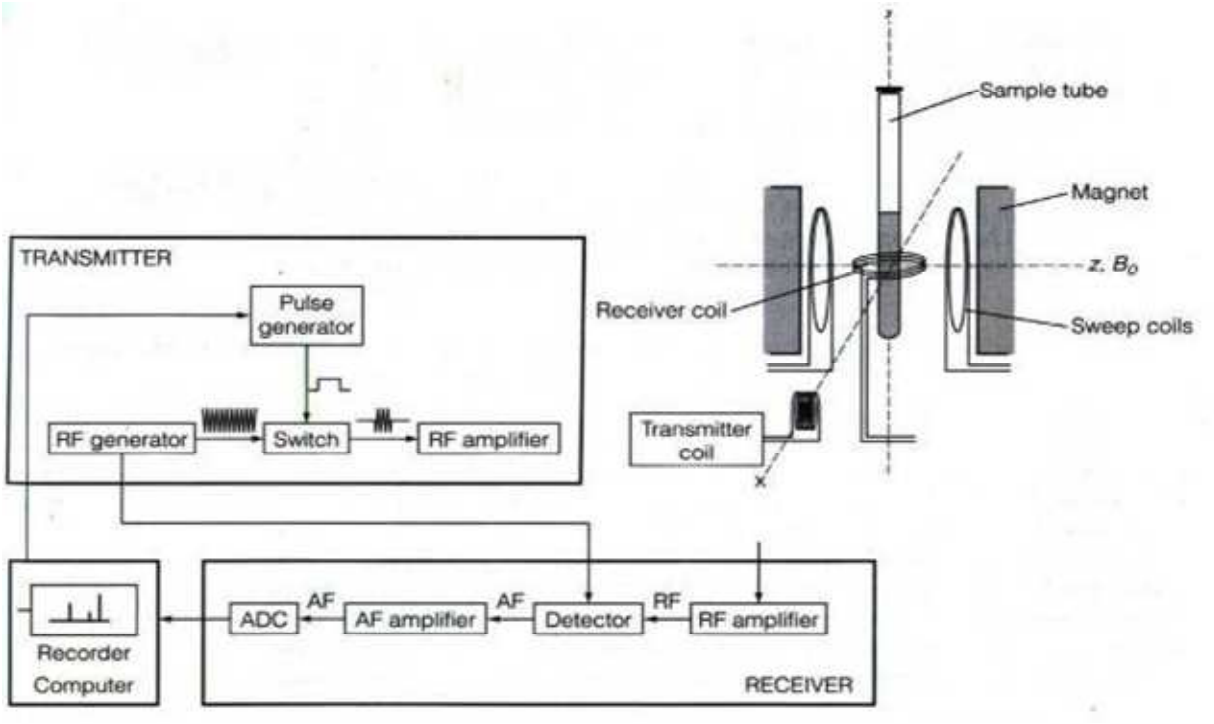


Fig. 3.1. Diagrammatic reprentation of an NMR spectrometer. *Reproduced from D. Kealey et al.(2002) Analytical Chemistry, BIOS Scientific Publishers Limited, Oxford, UK.*

The homogeneity and stability of the magnetic field should be at least 1 in 10^9 to ensure narrow absorption bands and good resolution. Sample tubes are made to spin in the sample probe at about 50Hz by an air turbine so as to increase the apparent field homogeneity further. The direction of the magnetic field and the orientations of the transmitter and receiver coils must be mutually perpendicular to detect sample resonances and eliminate spurious signals in the detector circuit as shown in Fig 3.1.

Stability of operation is improved considerably by locking the field and frequency together to correct for drift. This is achieved by constantly monitoring the resonance frequency of a

reference nucleus, usually deuterium in deuterated solvent. For carbon-13 studies in particular this is essential, as accumulating large numbers of scans can take several hours.

4.0 Conclusion

You would have learnt the principles of NMR spectroscopy, components of an NMR spectrometer and how an NMR spectrometer works.

5.0 Summary

- NMR involves absorption of electromagnetic (EM) radiation in the radiofrequency range by the nuclei of some isotopes.
- Absorption of the EM radiation when placed in a strong magnetic field can cause the nuclei in the lower energy parallel state to spin-flip to the higher energy antiparallel state. This is known as resonance.
- The absorption radiation is known as a signal
- Resonance can be brought about if the external field is kept constant and the radiofrequency varied and vice versa.
- Chemical shift is the ratio between the frequency of the signal and that of TMS (TMS) and the spectrometer operating frequency (MHz).
- A superconducting electromagnet provides a powerful magnetic field in NMR spectrometers
- The sample probe is positioned between the poles of the magnet.

6.0 Tutor-Marked Assignments

1. Explain the principles of NMR spectroscopy
2. What do you understand by field sweep and frequency sweep.
3. What is a chemical shift
4. Describe the different components of a NMR spectrometer.

7.0 References and Other sources.

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 248-260.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 163-171.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 176-178.

UNIT 2 PROTON MAGNETIC NUCLEAR RESONANCE

1.0 Introduction

Proton magnetic resonance (^{1}H-NMR or PMR) spectra are obtained using a solution of the sample containing a little TMS in a solvent that does not absorb in the radiofrequency region being studied. Tetrachloromethane (CCl_4) and deuterated solvents, such as deuterated trichloromethane ($CDCl_3$), methanol (CD_3OD) and propanone (CD_3COCD_3) are commonly used. These deuterated solvents all contain a small amount of the corresponding protonated compound because of incomplete deuteration during their manufacture. Deuterotrichloromethane ($CDCl_3$), for example, will contain a little trichloromethane ($CHCl_3$). Since ^{1}H-NMR spectra are additive, the signals of these impurities will occur in the spectrum and it is important that they are recognised and taken into account when interpreting a spectrum. The nature of the solvent will also affect the value of the chemical shift of a signal. Changing from tetrachloromethane to deutrotrichloromethane has little effect on the chemical shift, but changing to more polar solvents can cause a significant change in its value. These changes are sometimes used to help identify signals.

2.0 Objectives

You will know

- How a ^{1}H-NMR spectrum is generated
- The various solvents used in ^{1}H-NMR spectroscopy
- Understand the splitting pattern in ^{1}H-NMR spectroscopy
- How to apply coupling constants to give useful information in structure elucidation.
- How to determine the number of protons that correspond to a particular signal.

3.0 Main Content

3.1 ^{1}H-NMR Spectra

Most proton chemical shifts occur in the 0 to 12 ppm region. Identification is made in the first instance using broad correlation charts as shown in Fig. 3.2 below. This is followed by an estimate the chemical shift of a proton in a model compound and compare it with the value obtained in practice. If the comparison gives a good match it is taken as evidence that the signal on the spectrum is due to a similar type of structure.

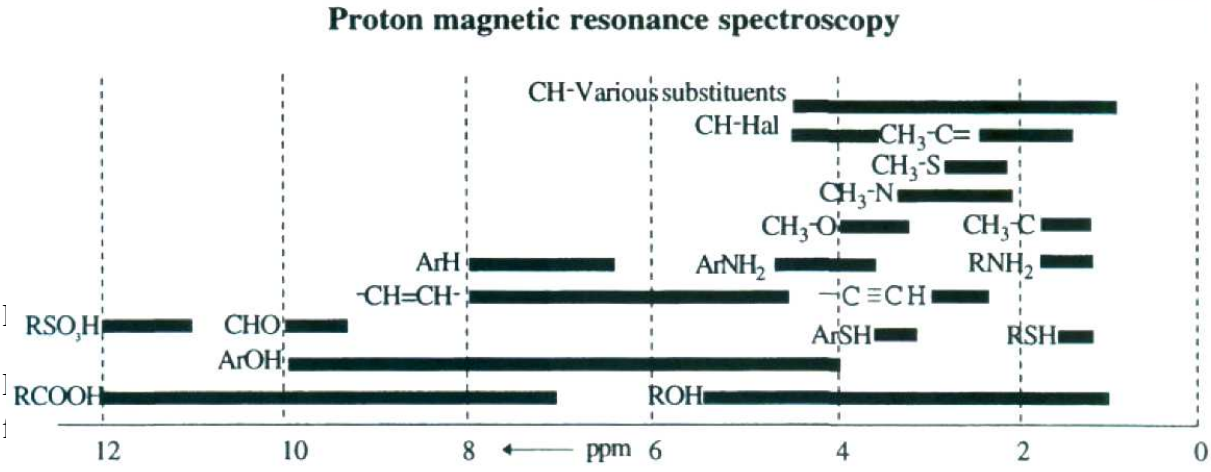


Fig. 3.2. A p. m. r. general correlation chart. *Reproduced from G. Thomas, Chemistry for Pharmacy and the Life sciences, Pearson Education Ltd, Essex, England, 1996*.

Protons that are in the same chemical and therefore in the same magnetic environment will have identical chemical shifts. They are known as equivalent protons e.g. the three protons of the methyl group of bromoethane will be equivalent and have the same chemical shift (Fig. 3.3)

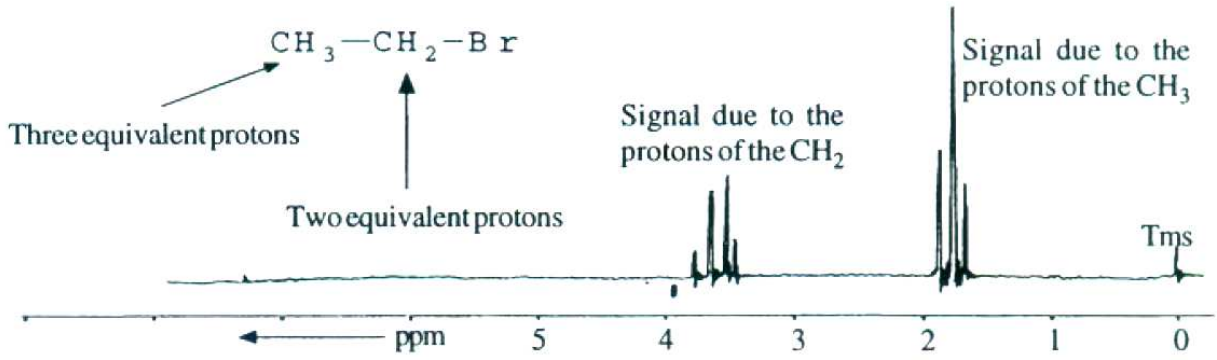


Fig. 3.3. Equivalent protons in bromoethane. .
Reproduced from G. Thomas, Chemistry for Pharmacy

Similarly, the two protons of the methylene group will also be equivalent because there is unrestricted free rotation about the C-C bond. However, it is not safe to assume that protons attached to the same atoms are always equivalent.

3.2 Spin-Spin Coupling

The signals seen on a ^{1}H-NMR spectrum vary from a single peak to a group of peaks. The division of a signal into the group of peaks occur because the magnetic fields of adjacent protons influence the magnetic field strength at which a proton comes into resonance. Consider, for example, the ^{1}H-NMR spectrum of a sample of dichloroethanal. For

convenience, the protons of this compound will be referred to as H_A and H_B respectively (Fig. 3.4)

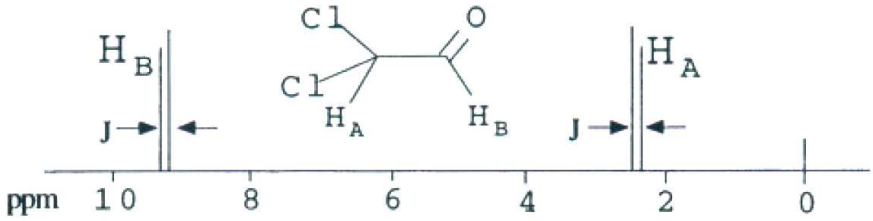


Fig. 3.4. The ^{1}H-NMR spectrum of dichloroethanal. . *Reproduced from G. Thomas, Chemistry for Pharmacy and the Life sciences, Pearson Education Ltd, Essex, England, 1996*.

The only other nuclei in dichloroethanal that have local magnetic fields that will affect the H_A protons is the proton H_B. As a result the spectrum shows a pair of peaks (a doublet) for the absorption signal of the proton H_A. Similarly, the signal of H_B will also be a doublet since it is affected in the same way by H_A. This behaviour is known as spin-spin coupling or splitting. The value of the chemical shift separating the peaks in each doublet is known as the coupling constant J. It is a constant characteristic of protons that are spin-spin coupled. In the ^{1}H-NMR spectrum of dichloroethanal, for example, H_A will have the same coupling constant as H_B. This enables one to pick out the signals of hydrogen atoms that are spin-coupled and so adjacent to each other in a structure, which is of considerable help in the interpretation of a spectrum.

It should be noted that:

a. Chemically equivalent protons do not couple with each other even if they are bonded to different carbon atoms.

b. Protons that are further than two single 'bond lengths' apart do not usually couple.

c. Protons that are spin-coupled with each other have the same J values.

More complex splitting patterns are observed when more than two protons are involved in the coupling. In theory, the number of peaks occurring in a signal will be n + 1, where n is the number of equivalent protons whilst their relative intensities are predicted by Pascal's triangle. Consider, for example, the ^{1}H-NMR spectrum of bromoethane (CH_3CH_2Br). Unrestricted free rotation about the C-C bond means that the three protons of the methyl group are equivalent and the two protons of the methylene group are equivalent. Therefore, in theory, the methyl protons with their two equivalent neighbouring protons will have a signal that is a triplet (2+1) with the intensities of the peaks in the ratio 1:2:1. On the other hand, the methylene group has three equivalent neighbours and so its signal will be predicted to be a quartet (3+1) with the peaks having relative intensities of 1:3:3:1. This agrees reasonably well with the spectrum of bromoethane which also shows that the signal for the

methyl's protons is upfield from that of the methylene (see Fig. 3.3) This type of prediction is reasonably accurate for simple molecules but less accurate for more complex molecules because the three-dimensional nature of these molecules sometimes makes it difficult to identify all the nuclei that can affect a signal.

n=0 1

n=1 1 1

n=2 1 2 1

n=3 1 3 3 1

n=4 1 4 6 4 1

Figure 3.5 Pascal's triangle

3.3 Signal Intensity

The area under a signal in a ^{1}H-NMR spectrum is proportional to the number of equivalent protons responsible for that signal. Consequently, electronic configuration of the area under each signal in a ^{1}H-NMR spectrum enables one to determine the ratio of the numbers of equivalent protons responsible for each signal. This can be of considerable help in interpreting ^{1}H-NMR.

3.4 Deuterium exchange

Deuterium does not absorb radiofrequency radiation in the same region as protons. However, it will undergo rapid exchange reactions with some acidic protons such as those in the hydroxyl and amino groups. The mixing of D_2O with a sample (known as D_2O shake) causes either a reduction in the intensity or the complete removal of the signals in a spectrum due to exchangeable protons. This loss will be accompanied by the appearance of a weak signal at 4.8 ppm produced by the formation of HOD provided the HOD is soluble in the solvent used. The changes in a spectrum caused by a D_2O shake enables one to identify the signals of groups that contain exchangeable protons.

3.5 Interpretation of Proton NMR Spectra

The following is a general guideline to ^{1}H-NMR spectral interpretation

a. Note the presence or absence of saturated structures, most of which give resonances between 0 and 5 ppm

b. Note the presence or absence of unsaturated structures in the region between about 5 and 9 ppm (alkene protons between 5 and 7 ppm and aromatic protons between 7 and 9 ppm, alkyne protons are an exception appearing at about 1.5 ppm).

Note any very low field resonances (9 to 16 ppm), which are associated with aldehydic and acidic protons, especially those involved in strong hydrogen bonding.

c. Measure the integrals, if recorded and calculate the numbers of protons in each resonance signal.

d. Check for spin-spin splitting patterns given by adjacent alkyl groups according to the n +1 rule and Pascal's triangle. (The position of the lower field multiplet of the two is very sensitive to the proximity of electronegative elements and groups such as O, CO, COO, OH, Cl, Br, NH_2 etc).

e. Examine the splitting pattern given by aromatic protons, which couple around the ring and are often complex due to second order effects.

f. 1,4- and 1,2-disubstituted rings give complex but symmetrical looking patterns of peaks, whereas mono-, 1,3-and tri-substituted rings give more complex asymmetrical patterns.

e. Note any broad single resonances, which are evidences of labile protons form alcohols, phenols, acids and amines that can undergo slow exchange with other labile protons. Comparison of the spectrum with another after shaking the sample with a few drops of D_2O will confirm the presence of an exchangeable proton by the disappearance of its resonance signal and appearance of another at 4.7 ppm due to HOD.

3.6 EXAMPLES OF ^{1}H-NMR SPECTRAL INTERPRETATION

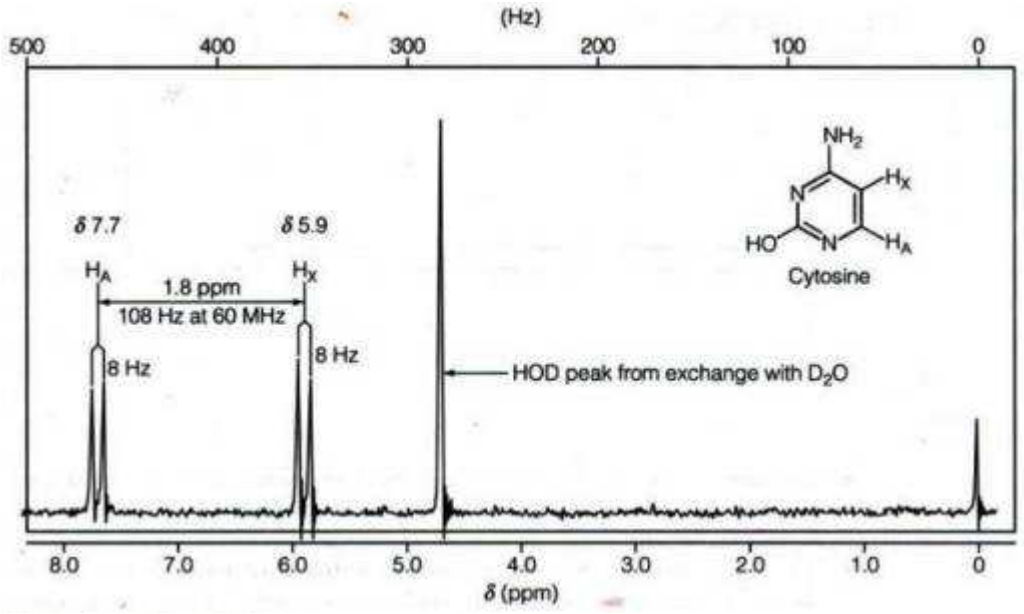


Fig. 3. 6: ^{1}H Spectrum of cytosine. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

The two aromatic protons, A and X, in cytosine are coupled to give an AX pattern of two doublets. The A proton is deshielded more than the X proton due to its closer proximity to nitrogens and the oxygen atom. The intensities of the doublets are slightly distorted by second order effects. The OH and NH_2 protons have been exchanged with D_2O, and their resonance replaced with a HOD peak at 4.7ppm.

Another example is in Fig.3.7 below

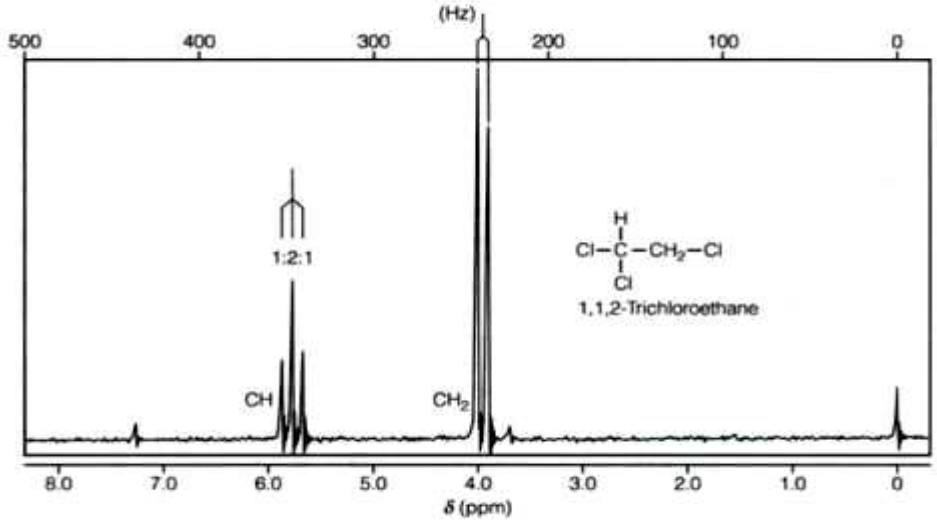


Fig. 3.7: ^{1}H-NMR spectrum of 1,1,2-trichloroethane. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

The CH (methane) resonance in 1,1,2-trichloroethane is at a much lower field than the CH_2 (methylene) resonance because of the very strong deshielding by two chlorines . The protons give an AX_2 coupling pattern of a triplet and a doublet and an integral ratio of 1:2.

4.0 Conclusion

You should now know how to prepare samples for ^{1}H-NMR spectroscopy, what a ^{1}H-NMR spectrum looks like and how to interpret a ^{1}H-NMR spectrum.

5.0 Summary

- ^{1}H-NMR spectra are obtained using a solution of the sample in deuterated solvents such as $CDCl_3$, CD_3OD containing a little TMS.
- Most proton chemical shifts occur in the 0 to 12 ppm region
- ^{1}H-NMR correlation charts can be used to interpret ^{1}H-NMR spectra.
- Protons in the same chemical environment have identical chemical shifts
- Splitting of signals into groups of peaks occur because the magnetic field of adjacent protons influence each other. This is known as spin-spin coupling or splitting.

- The area under a signal in a ^{1}H-NMR spectrum is proportional to the number of equivalent protons responsible for that signal.
- OH and NH_2 protons can be exchanged with D_2O. This helps in easy identification of these functional groups.

6.0 Tutor-Marked Assignments

1. Define the term 'coupling constant'
2. Mention how a sample is prepared for ^{1}H-NMR experiment
3. Explain what you understand by spin-spin coupling
4. Mention the data that can be obtained from ^{1}H-NMR experiments that are needed in determining the structural features of an organic compound.
5. Assign the peaks in the spectrum below:

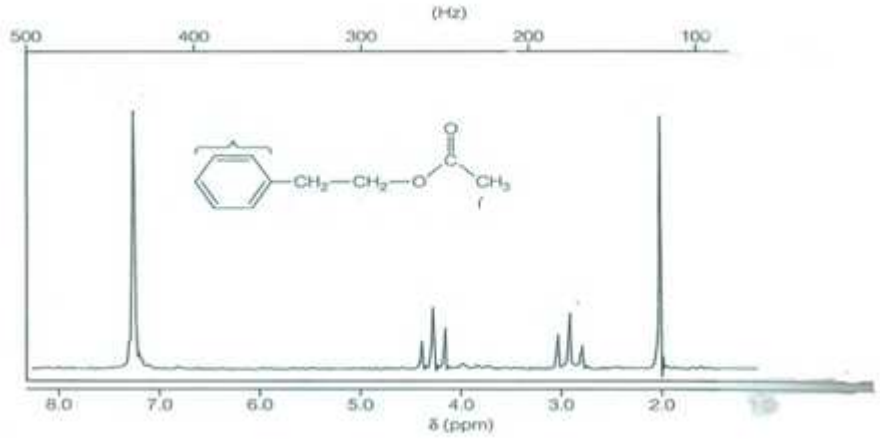


Figure 3.8: ^{1}H-NMR spectrum of Phenylethylethanoate. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

7.0 References and Other Sources

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed)*. 261-265.

2. Watson D.G. (2005) Pharmaceutical Analysis. *Elsevier Chruchill Livingstone, UK* (*2nd ed*). 163-171.

3. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed)*. 176-178.

UNIT 3 ^{13}C NUCLEAR MAGNETIC RESONANCE

1.0 Introduction

The origins and interpretation of ^{13}C nuclear magnetic resonance spectra are similar to proton magnetic resonance spectra. However, they are usually simpler and therefore easier to interpret. This is due to two main factors: (1) the low natural abundance of the isotope and (2) the mode of operation of the spectrometer. Both these factors simplify the spectrum by preventing the splitting of signals by spin-spin coupling between nuclei.

The natural abundance of ^{13}C isotopes is 1.1%, that is, roughly one carbon atom in every hundred is a ^{13}C isotope. This means that it is highly unlikely that adjacent carbon atoms in a molecule will be ^{13}C isotopes. Since ^{13}C nuclei do not absorb radiofrequency radiation when placed in a strong magnetic field, most of the carbon atoms in a molecule will be incapable of spin-spin coupling with any ^{13}C nuclei present. However, there will be sufficient ^{13}C isotopes in all the molecules found in a sample to obtain an average absorption spectrum of all the molecules in that sample. This average spectrum is in effect that of a theoretically impossible molecule whose carbon atoms are all non-coupled to ^{13}C isotopes

2.0 Objectives

- How a ^{13}C-NMR spectrum is generated
- Become familiar with the typical chemical shifts of some ^{13}C atoms.
- How to interpret ^{13}C spectra.

3.0 Main Content

3.1 ^{13}C-NMR Spectra

^{13}C resonance occurs at a frequency of ~ 25.1 mHz when proton resonance is occurring at ~ 100 mHz. Thus it is at lower energy than proton resonance and the spread of resonances for ^{13}C is over ca 180 ppm; thus there is less likelihood of overlapping lines in ^{13}C NMR. Figure 3.9 shows the chemical sifts of some ^{13}C signals. This is only an approximate guide

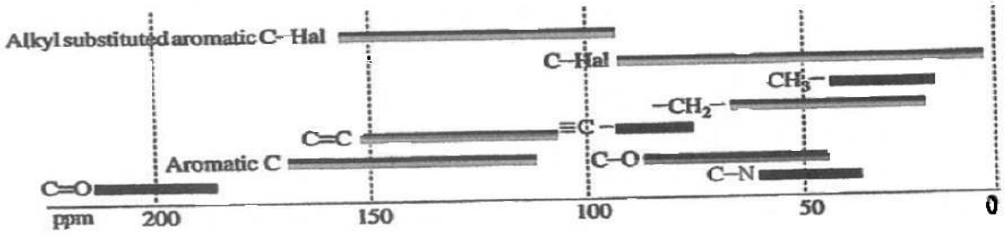


Figure 3.9: Typical Chemical Shifts of ^{13}C atoms. *Reproduced from G. Thomas. (1996) Chemistry for Pharmacy and the Life sciences. Pearson Education Ltd, Essex, England.*

A ^{13}C atom will couple to any protons attached to it e.g. a carbon with one proton attached will appear as a double. However to get the most information from the weak carbon spectrum, it is better if this coupling is removed.

Carbon-13 spectra cover a much wider range of chemical shifts than proton spectra, but the positions of resonances are generally determined by the same factors. However, for ease of interpretation, they are often recorded as decoupled spectra to eliminate the effects of coupling to adjacent protons which would otherwise split the carbon-13 resonances according to the n + 1 rule and Pascal's triangle. Decoupled spectra consists of a single peak for each chemically different carbon in the molecule and spectra interpretation is confined to the correlation of their chemical shifts with structure, augmented by reference to chemical shift data and the spectra of known compounds. Proton coupling can be observed under appropriate experimental conditions.

3.2 Interpretation of ^{13}C-NMR Spectra

The following is a general approach:

a. Note the presence or absence of saturated structures, usually give resonances between 0 and 90 ppm.

b. Note the presence or absence of unsaturated structures in the region between about 100 and 160 ppm. (alkyne carbons are an exception appearing between 70 and 100 ppm).

c. Note any very low field resonances (160 to 220 ppm), which are associated with carbonyl and ether carbons. Carboxylic acids, anhydrides, esters, amides, acylhalides and ethers are all found in the range 160 to 180 ppm, whilst aldehydes and ketones lie between 180 and about 220 ppm.

3.3 Examples of ^{13}C-NMRspectra interpretation.

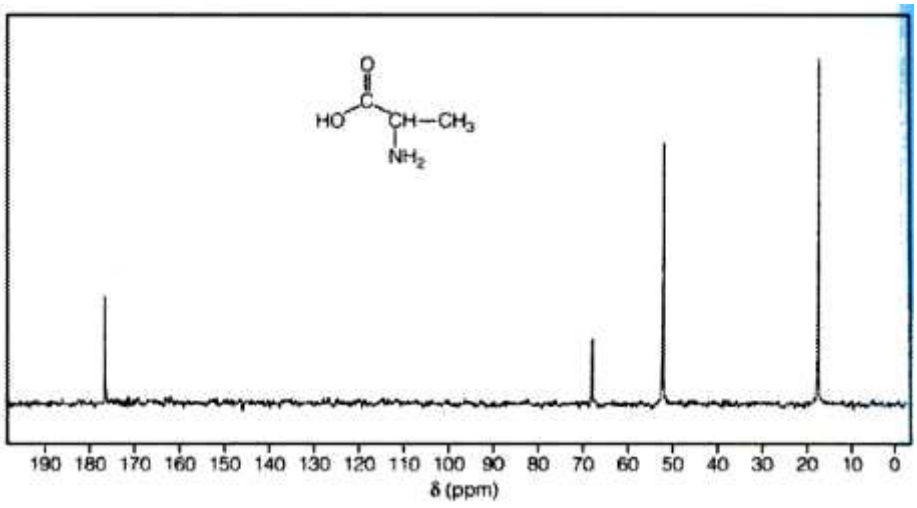


Fig.3.10 ^{13}C spectrum of alanine. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

The three carbons show a very wide range of chemical shifts. The lowest field resonance corresponds to the carbonyl carbon, which is highly deshielded by the double-bonded oxygen. The nitrogen deshields the CH carbon much less, and the CH_3 carbon is the least deshielded of the three.

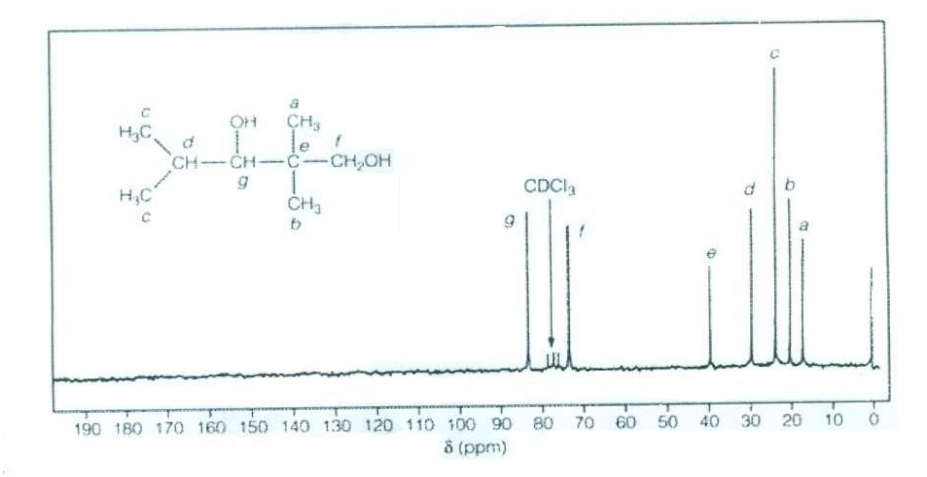


Figure 3.11 ^{13}C spectrum of 2,2,4-trimethyl-1,3-pentanediol. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

The carbon-13 resonances of this fully saturated compound are all found between 0 and 90 ppm. The two carbons directly bonded to oxygens are deshielded significantly more than the CH carbon, which in turn is more deshielded than the CH_3 carbons.

4.0 Conclusion

You should now know what a ^{13}C NMR spectrum looks like, and be able to interpret ^{13}C-NMR spectra.

5.0 Summary

- ^{13}C NMR spectra are simpler and easier to interpret due to the low abundance of the isotope and the mode of operation of the spectrometer.
- 13C atom will couple to protons attached to it, however 1H-decoupled experiments are usually performed for clarity.
- 1H-coupled experiments can be carried out under appropriate conditions.
- Saturated structures give resonances between 0 and 90 ppm
- Unsaturated structures give resonances between 100 and 160 except alkynes which resonate between 70 and 100 ppm.

6.0 Tutor-Marked Assignments

1. What is the difference between a ^{1}H-coupled ^{13}C-NMR spectra and ^{1}H-decoupled ^{13}C-NMR spectra.
2. Why are ^{13}C-NMR spectra generally less complex compared to ^{1}H-NMR spectra.
3. Mention some guidelines in interpreting ^{13}C-NMR spectra.
4. The ^{13}C-NMR spectra of 3-hydroxymethylbenzene is given below
   a) was the data generated from a ^{1}H-coupled or decoupled experiment?
   b) identify the carbon atom that corresponds to each signal.

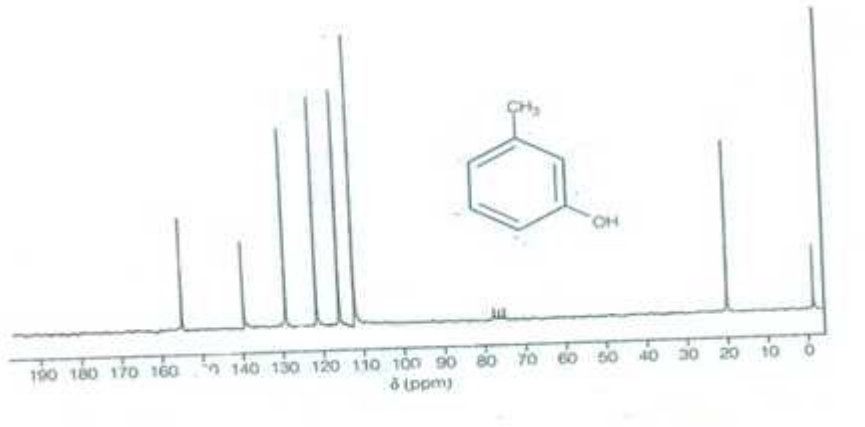


Figure 3.12: ^{13}C-NMR spectra of 3-hydroxymethylbenzene. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

7.0 References and Other sources

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 261-265.

2. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 176-178.

UNIT 4 TWO DIMENSIONAL NMR AND OTHER APPLICATIONS OF NMR

1.0 Introduction

Nuclear magnetic resonance spectroscopy is a powerful technique for the characterisation of the exact structure of compounds. This unit describes the use of two dimensional NMR in structure elucidation and other specialised application of NMR.

2.0 Objectives

You will know

- The importance of 2D-NMR
- how to interprete 2D-NMR data
- how NMR can be used in quantitative analysis
- other specialised applications of NMR

3.0 Main content

3.1 Two-dimensional NMR Spectra

^{1}H-^{1}H correlation or COSY experiments allow protons to be coupled to each other. Fig 3.12 shows the correlated spectroscopy spectrum of aspirin. The diagonal gives the correlation of the signals themselves i.e. A with A, B with B etc. On either side of the diagonal, identical information is presented; thus only one diagonal is required for spectral interpretation. From the spectra, it can be seen that A is coupled to C, B is coupled to C and D; and C is weakly coupled to D via long-range meta-coupling. COSY has simplified the interpretation of complex NMR spectra. There are a number of techniques stemming from the basic two-dimensional technique, which for example, allow correlation between carbon atoms and the protons attached to them and correlations of carbon atoms with protons one or two bonds removed from them, heteronuclear multiple bond correlation (HMBC).

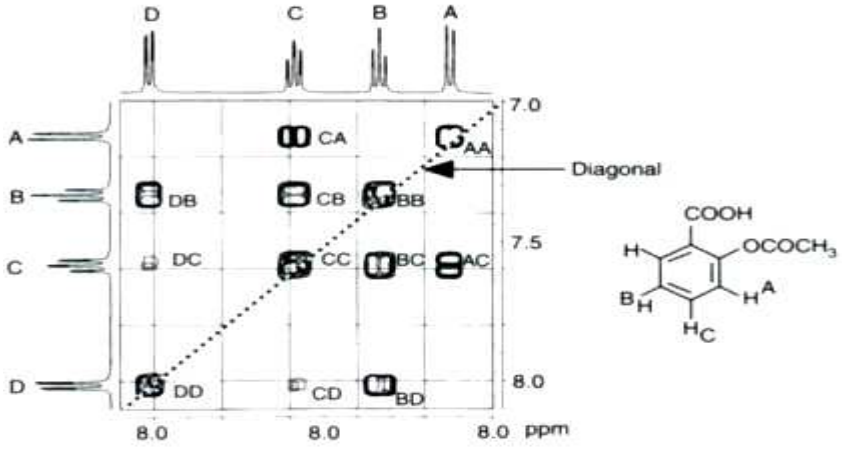


Figure 3.13: ^{1}H-^{1}H-COSY Spectrum of aspirin. *Reproduced from D.G. Watson. (2005) Pharmaceutical Analysis. Elsevier Churchill Livingstone, UK.*

3.2 Other applications of Nuclear Magnetic Resonance Spectroscopy

We have discussed the use of NMR as a powerful technique for the characterisation of the exact structure of organic compounds using ^{1}H-NMR, ^{13}C-NMR and COSY experiments. Other applications include:

a) determination of impurities including enantiomeric impurities without separation down to ca 10% level.

b) NMR can be used as a rapid and specific quantitative technique. For example, a drug can be rapidly quantified by measuring suitable protons (often isolated methyl protons) against the intense singlet for the methyl groups in t-butanol. The amount of drug present can be calculated using the following formula for the methyl groups in t-butanol used as an internal standard (int. std.)

$$\text{Amount of drug} = \frac{\text{Area signal for drug protons}}{\text{Area signal for int.std.protons}} \times \text{mass of int. std. added} \times \frac{\text{MW drug}}{\text{MW int.std}} \times \frac{\text{No of protons from int.std}}{\text{No of protons from drug}}$$

An advantage of this method of quantitation is that a pure external standard for the drug is not required since the response is purely proportional to the number of protons present and this can be measured against a pure internal standard. Thus, the purity of a substance can now be determined without a pure standard for it being available.

c. NMR has been developed recently, in conjunction with chemometrics, as a tool for the diagnosis of disease. For these purposes it is often interfaced with HPLC. It has also found wide application in magnetic resonance imaging, a technique that may be used for imaging soft tissues using NMR signal produced by the protons within the tissues. LC-NMR has also found application in drug metabolic studies.

4.0 Conclusion

In this unit you have learnt about two dimensional NMR experiments and how the data can be used to generate useful information in structural elucidation. You have also learnt that NMR can be used in quantitative analysis and in diagnosing a disease.

5.0 Summary

- The diagonal in correlated spectroscopy gives the correlation of the signals.
- Only one side of the diagonal is required for spectral interpretation.
- NMR can be used in quantitative analysis of drug molecules by measuring suitable protons (often isolated methyl groups) against the intense singlet for the methyl groups in t-butanol.
- An external standard is not required to determine the purity of a drug.

- NMR can be coupled to HPLC and this has been used in diagnosing a disease.

6.0 Tutor-Marked Assignments

1. Write short notes of two dimensional NMR (COSY)
2. Mention two specialised application of NMR
3. Explain how NMR can be used in quantitative analysis.

7.0 References and Other Sources

1. Thomas G. (1996) Chemistry for Pharmacy and the Life sciences. *Pearson Education Lts, Essex, England (1st ed).* 180-185.

UNIT 5 STRUCTURE ELUCIDATION OF ORGANIC MOLECULES WITH WORKED EXAMPLES

1.0 Introduction

The information that may be obtained from ultraviolet-visible, infrared, proton and carbon-13 nuclear magnetic resonance and mass spectra is complementary, and it is much easier to identify the structure of a compound if all the spectra are considered. Each spectrometric technique provides characteristic data to assist in the eventual identification of the sample. These have been considered in the individual topics, but need to be combined to extract the maximum information.

2.0 Objectives

You will be able to

- Appreciate how to use all the data generated from the various spectroscopic method to determine the structure of a compound

3.0 Main content

If an unknown material is presented for analysis, it should first be determined whether the sample is a single substance, or a mixture. The purity of substances such as pharmaceuticals is very important. Separation by an appropriate technique should reveal the number of components in the sample.

3.2 Information from each spectrum

In order to study and identify any unknown analytical sample using spectroscopic techniques, the analytical chemist must first obtain good quality spectra and then use these to select the information from each technique that is of most value. It is also important to recognize that other analytical observations should be taken into account. For example, if the sample is a volatile liquid and the spectral information suggests that it is an involatile solid, clearly, there is conflicting evidence.

The use of computerized library databases can assist in the matching of spectra to recorded examples. If difficulties are found in distinguishing between two possibilities for the sample identity, then it may be necessary to consult reference texts or additional computer databases so that an exact match is found. Some databases give information that helps when working with samples that are new compounds or whose spectra are not present in the database. For example, the presence of a strong peak in an IR spectrum near 1700 cm^{-1} should suggest a high probability that the sample might be a carbonyl compound.

3.3 Spectroscopic identification

The conditions under which each spectrum has been obtained must be taken into consideration. For example, if the UV, IR and NMR spectra were run in solution, what was

the solvent? The instrumental parameters also need to be considered. In MS, the type of ionisation used will affect the spectrum obtained.

If the source of the analytical sample is known, this can be a great help in elucidating the identity of the material. It is a worthwhile exercise to follow the same general scheme and to note down the information that is deduced form the study of each spectrum. One suggested scheme is given below, but the value of 'feedback' in checking the deductions must not be overlooked.

i) Empirical formula

Occasionally, if the sample has been analyzed to find the percentage of carbon, hydrogen, nitrogen, sulphur and other elements, and to deduce the percentage of oxygen by difference, this can be a useful first step. If this information is not available, it may be found from the MS if an accurate relative molecular mass has been measured.

Example: A solid sample contained C 75.5%, H 7.5% and N 8.1% by weight. What is the empirical formula of the sample?

Dividing by the relative atomic masses gives the ratio of numbers of atoms, noting that there must be (100-75.5-75.1-7.5-8.10) = 8.9% oxygen.

C = 75.5/12 =6.22

H = 7.5/1 = 7.5

N = 8.1/14 = 0.578

O = 8.9/16 = 0.556

This corresponds (roughly) to $C_{11}H_{13}NO$, with an RMM of 175, which may give a molecular ion in the mass spectrum.

ii) Double bond equivalents.

The presence of instauration in a structure should be considered. Since a saturated hydrocarbon has the formula C_nH_{2n+2}, and since a single-bonded oxygen can be thought of as equivalent to $–CH_2-$, and a single-bonded nitrogen as –CH<, the number of double bonds or rings, called the double bond equivalents (DBE) for the compound is given by:

$$DBE = (2n_4 + 2 + n_3 - n_1)/2$$

Where n_4 is the number of tetravalent atoms (e.g. carbon), n_3 is the number of trivalent atoms (e.g. nitrogen), 1 is the number of monovalent atoms (e.g. hydrogen or halogen).

Therefore, for benzene, C_6H_6, the DBE is (14-6)/2, that is , three double bonds and one ring.

For example in (i) above, $C_{11}H_{13}NO$, the DBE is (24 + 1-13)/2 = 6, which would correspond to one benzene ring (4) plus one –C=C- plus one >C=O. Note that other spectra must be used to distinguish between a ring and a double bond or between a –C=C- and a >C=O.

iii) The IR spectrum gives evidence about the presence of functional groups discussed in module 1. The example in (ii) above would be solved if the infrared spectrum showed no carbonyl to be present. The presence of aliphatic groups, or unsaturated or aromatic structures may be inferred from the position of the –C-H stretching bands around 3000 cm^{-1}, and confirmed by the presence of other bands. A useful application of Raman spectrometry is the detection of groups that have very weak absorbances in the infrared region, such as substituted alkynes.

iv) The UV spectrum does give some structural information, even when there is little or no absorbance, which would suggest the absence of any aromatic, conjuagated or ketonic structures. If there are double bonds or unsaturated rings present, the UV spectrum should provide further information.

(v) Much useful information may be derived from the mass spectrum as discussed fully in the module 2. A brief summary of what you should look for should include:

- The m/z of the molecular ion. This corresponds to the molecular formula, which may be a multiple of the empirical formula derived in (i). An odd value for the m/z of the molecular ion requires that an odd number of nitrogen atoms are present, as in the example in (i) above. Prominent isotope peaks indicate the presence of Cl, Br or S.
- The exact value of m/z of the molecular ion. For example, the nominal RMM of the example in (i) is 175, and the formular might have been deduced if the exact mass was determined as 175.0998, since, excluding some impossible formulae, some others are:

  $C_8H_5N_3O_2$ 175.0382
  $C_7H_{13}NO_4$ 175.0845
  $C_{11}H_{13}NO$ 175.0998
  $C_{10}H_{13}N_3$ 175.1111
- The fragments present and the fragments lost.

(vi) Both the ^{1}H-NMR and the ^{13}C-NMR give essential information about the types of protons and carbons present, their environment and their connections to neighbouring atoms. This is discussed in detail in Units 2 and 3 of this module.

(vii) Before the final report is given, it is always a good idea to retrace the steps above the check whether the data is self-consistent. For example, if there is no evidence for aromatic structures in the IR spectrum, is this consistent with the NMR spectrum? If an isomer must be identified, do the positions of the peaks in the IR and NMR spectra correspond, and does the fragmentation in the mass spectrum provide confirmation?

3.3 Applications

Example 1:

The spectra shown in Figure 1 (a)-(d) were obtained for a compound of composition C 67%, H 7.3%, N 7.8%, melting at 135ºC

(i) Empirical formula: $C_{10}H_{13}N_2$; RMM = 179

(ii) DBE = 5

(iii) 1a:IR (KBr disk)

3300 cm-1	H-N stretch
3000+	H-C aromatic stretch
3000	H-C aliphatic
1670	C=O stretch (amide or aromatic links?)
1650, 1510, etc	aromatic ring vibrations

This suggests a substituted aromatic amide.

(iv) UV (methanol solution): major peaks at 243 and 280 nm also suggest an aromatic compound.

(v) 1b: MS (EI)

m/z	
179	$M^{+\bullet}$ must be odd number of nitrogens
137	M- 42: loss of CH_2CO; CH_3CO- compound?
43	CH_3CO^+
27 and 29	$C_2H_3^+$ and $C_2H_5^+$
108/109	$(OH\text{-}C_6H_4\text{-}NH_2)^+$ and less 1H

(vi) 1c: ^{1}H NMR (80MHz, CCl_4 solution)

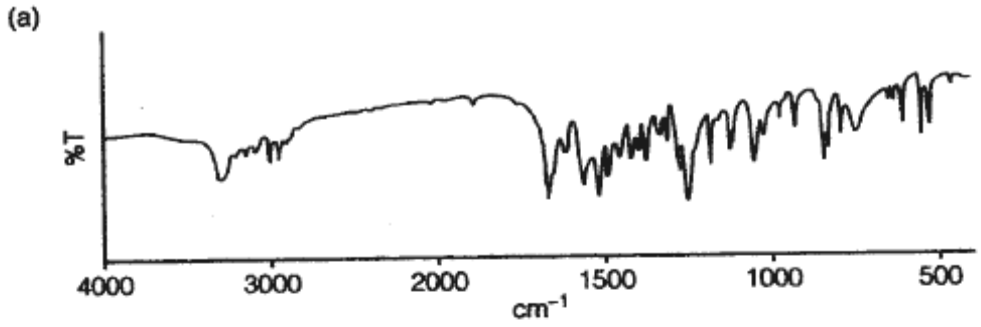
∂/ppm	Relative integral	Multiplicity	Assignment

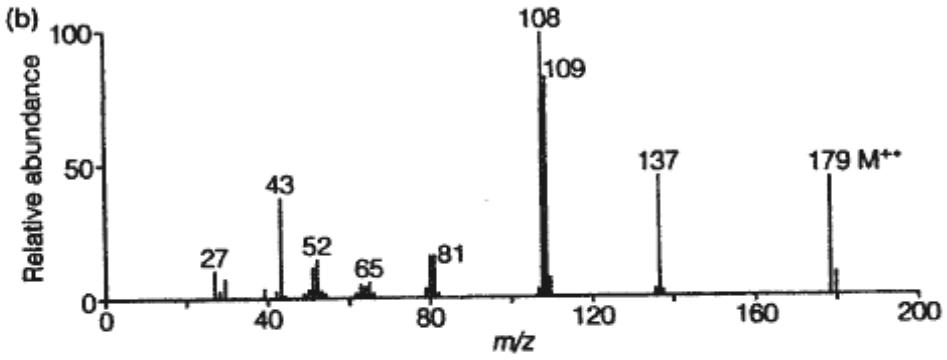
1.3	3	3	CH_3-CH_2-
2.1	3	1	CH_3-CO-
4.0	2	4	O-CH_2-CH_3
6.8/7.3	4	~2 doublets	1,4-ArH-
7.6	1	1, broad	Ar-NH-CO

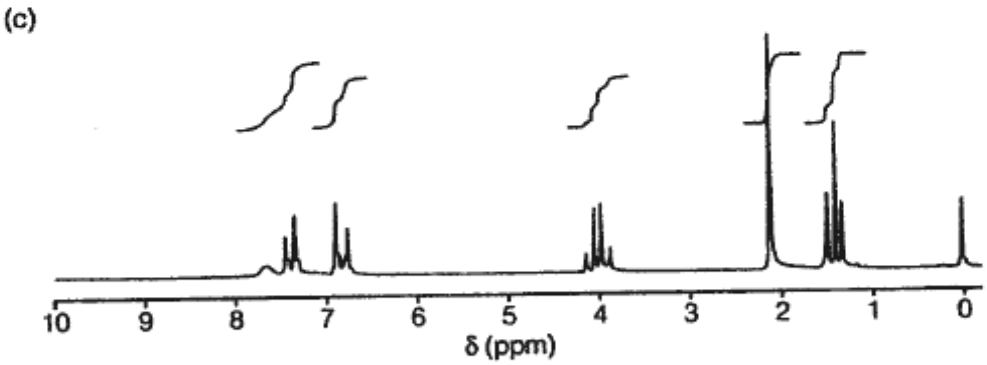
1d: ^{13}C-NMR (20.15 MHz, $CDCl_3$ solution)

□/ppm	Multiplicity	Assignment
14.8	4	CH_3-CH_2-
24.2	4	CH_3-CO-
63.7	3	O-CH_2-CH_3
114.7	2	ArCH-
122.0	2	ArCH
131.0	1	ArC-CO-
155.8	1	ArC-N-
168.5	1	Ar-CO-

(vii) The pair of doublets in the proton NMR suggests a 1,4-disubstituted aromatic compound. Evidence for an ethyl group and for an amide suggest the structure C_2H_5O-C_6H_4-$NHCOCH_3$, 4-ethoxyacetanilide (phenacetin).


(a)
%T
4000
3000
2000
1500
1000
500
cm⁻¹


(b)
Relative abundance
100
50
0
108
109
137
179 M+•
43
27
52
65
81
0
40
80
120
160
200
m/z


(c)
10
9
8
7
6
5
4
3
2
1
0
δ (ppm)

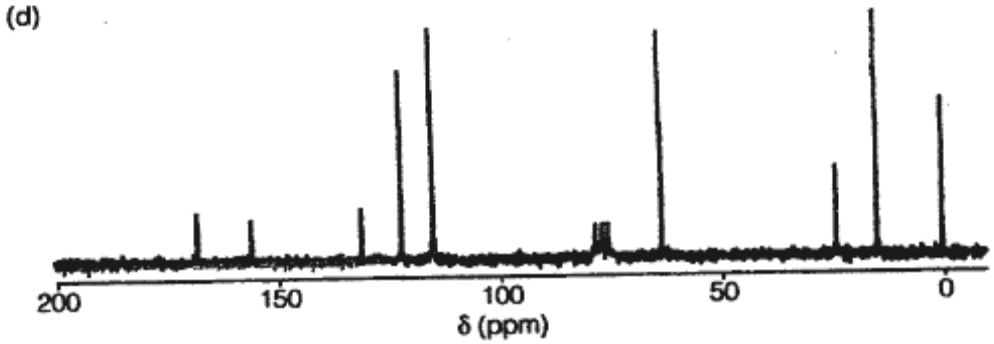

(d)
200
150
100
50
0
δ (ppm)

Figure 3.14: Example 1. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

Example 2

The spectra shown in Fig 3.15 (a)-(d) are for a liquid at 141ºC and soluble in water. The elemental composition was C 48.6%, H 8.1%.

(i) Empirical formula: $C_3H_6O_2$; RMM = 74

(ii) DBE = 1

(iii) 2a: IR (liquid film) The most notable features of the spectrum are the broad band around 3000 cm^{-1} and the strong carbonyl band at 1715 cm^{-1}.

3000cm-1	H-O-, hydrogen bonded stretch of acid
2900	H-C-aliphatic stretch
1715	C=O stretch of an acid
1450	CH_2 and CH_3 bend
1380	CH_3 bend
1270	C-O- stretch

This strongly suggests a carboxylic acid.

(iv) UV: No significant UV absorption above 220 nm, therefore aliphatic.

(v) 2b: MS (EI)

m/z	
74	$M^{+\bullet}$
57	M- 17 possibly M-OH
45	-COOH
27, 29	C_2H_5 present?

The fragment ions suggest an aliphatic carboxylic acid

(vi) 2c: ^{13}C-NMR (200 MHz, $CDCl_3$ solution)

∂/ppm	Multiplicity	Assignment
9.5	4	CH_3-C
28.2	3	-CO-CH_2-C

180.0 1 -CO-

(vii) The compound is propanoic acid, CH_3-CH_2-COOH. This is in agreement with all the spectroscopic data, and with the boiling point.

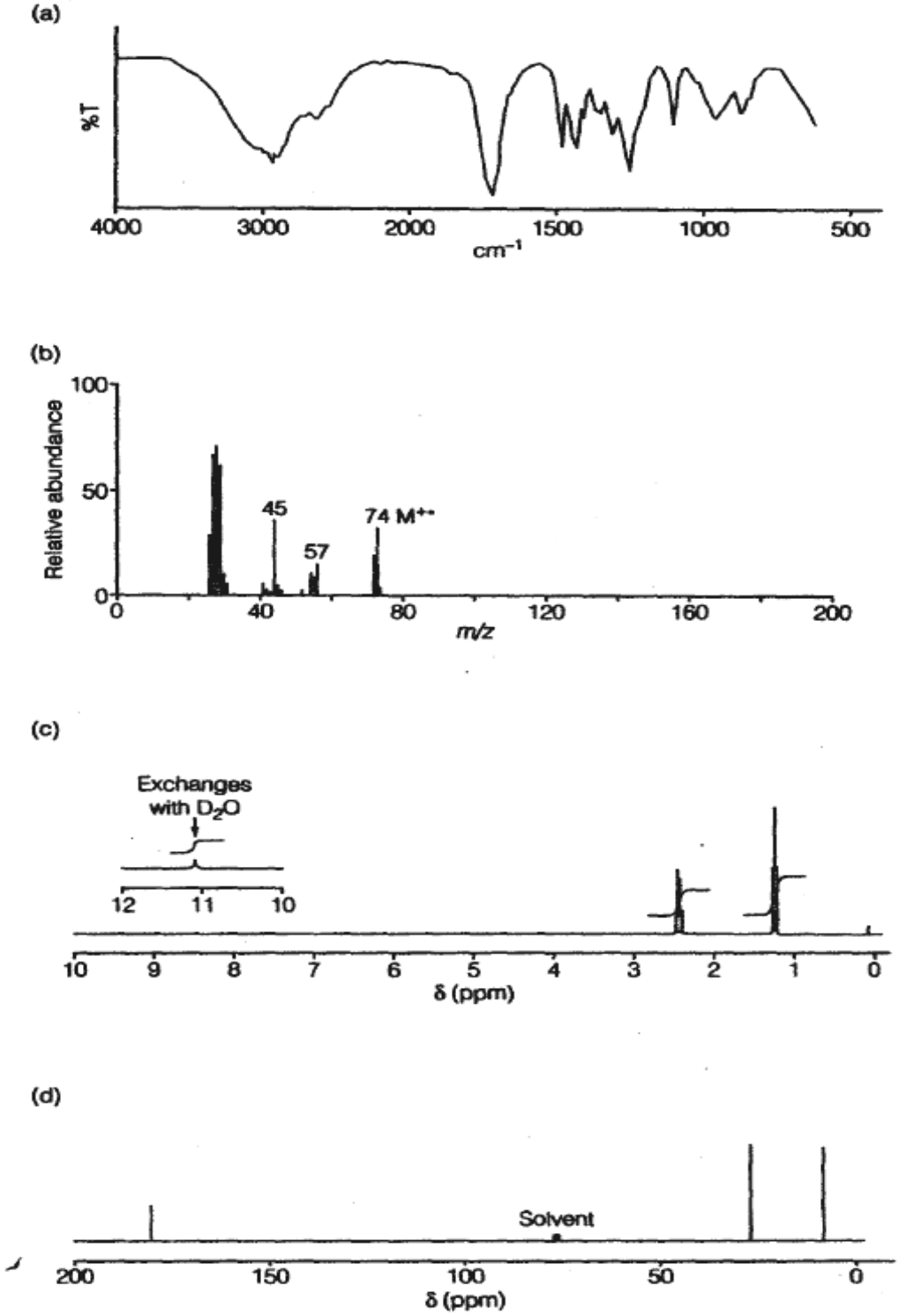


Figure 3.15: Example 2. *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

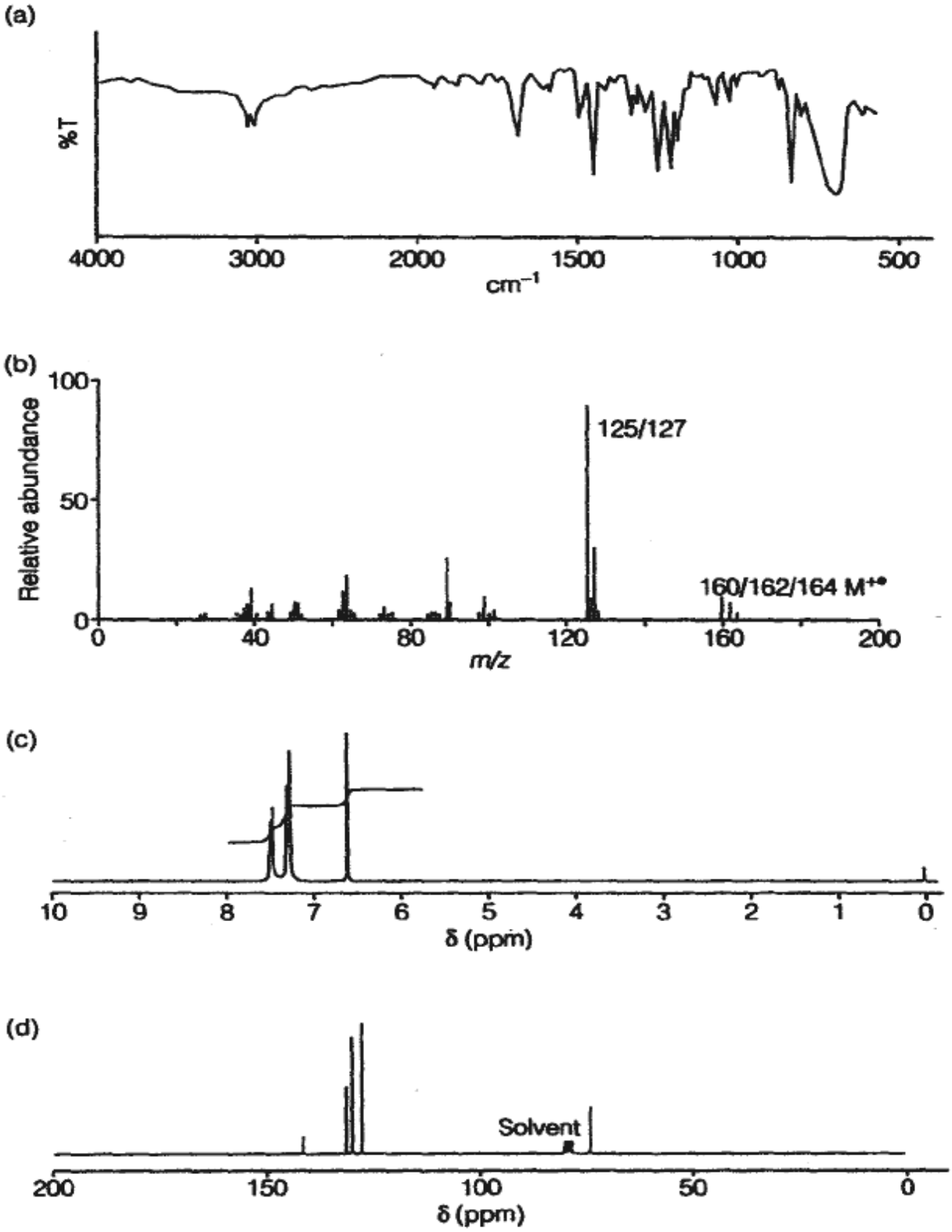


Figure 3.16: *Reproduced from D. Kealey et al.,(2002) Analytical Chemistry. BIOS Scientific Publishers Ltd, Oxford.*

4.0 Conclusion

From what you have learnt in this unit, you can now understand that multiple spectroscopic techniques is required for accurate determining the molecular structure of organic molecules.

5.0 Summary

- Good quality spectra is required in identification of a sample.
- Other analytical observations such as boiling point, melting point should be taken into account in sample identification.
- Each technique provides characteristic data that assist in the eventual identification of the sample.
- The combined approach often provides a better picture and it is more reliable than when using individual technique in isolation.

6.0 Tutor-Marked Assignment

The spectra shown in Figure 3.16 (a)-(d) are for a compound boiling at 205º and insoluble in water. The composition is C 52.2%, H 3.7%, Cl 44.1%.

a) Determine the empirical formula and DBE of the compound

The compound has a weak UV absorbance at 270 nm.

Using all the spectral data given in Figure 3.13, determine the molecular structure of the compound.

7.0 References and Other sources

1. Kealey D., Haines P.J. (2002) Analytical Chemistry. *BIOS Scientific Publishers Limited, Oxford,UK (1st ed).* 261-265.

2. Field L.D., Sternhell S., Kalman J.R. (2002) Organic Structures from Spectra. John Wiley & Sons Ltd, West Sussex, UK (3rd eds.) 367pp.