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Essential Tools for Organic Compounds Characterization: An Overview

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Abstract: Nuclear magnetic resonance (NMR) spectrometers, mass spectrometers, Fourier transform infrared (FTIR) spectrometers, UV-visible spectrophotometers, gas chromatography, and high performance liquid chromatography are the main tools used for characterization. GC-MS, LC-MS, X-ray diffraction (XRD), polarimeter, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) with EDX are examples of advanced and specialized instruments and Thin Layer Chromatography (TLC) is a common chromatography technique. The atomic environment and carbon-hydrogen connection are determined using a nuclear magnetic resonance (NMR) spectrometer. The molecular weight, structure, and fragmentation patterns are identified using a mass spectrometer (MS). A Fourier Transform Infrared (FTIR) Spectrometer is used to determine whether a molecule has functional groups. Electronic transitions and conjugation are analyzed using a UV-visible spectrophotometer. Heat-stable, volatile mixtures are separated using gas chromatography (GC). Complex non-volatile mixtures are separated and quantified using High-Performance Liquid Chromatography (HPLC). Compounds that have been isolated are identified using GC-MS and LC-MS. X-ray diffraction (XRD) is used to determine crystalline structure. Optical activity is measured with a polarimeter. Stability and thermal characteristics are examined using thermal analyzers (DSC/TGA). For single particle examination, a scanning electron microscope (SEM) equipped with EDX is utilized. Small-scale purification is another application for Thin Layer Chromatography (TLC), but its main purpose is characterisation. While preparative TLC can separate small amounts of compounds, it is a quick analytical tool that verifies the quantity of components in a mixture and their Rf values.

Keywords: HPLC, XRD, MS, GC, NMR, UV-VIS spectrophotometer.

I. Introduction

Spectrophotometric Methods [1,2]

1. Ultra-violet and Visible Spectrophotometry

Analytical methods based on the detection of light absorption by substances between 190 and 900 nm are collectively referred to as ultraviolet (UV) and visible absorption procedures. The ultraviolet (UV) section of the spectrum spans from 190 to 380 nm, whereas the visible (visible) area is from 380 to 900 nm. The visible spectrum (380 nm - 900 nm) is the origin of UV-visible absorption. Molecular electronic transitions are the source of UV-Visible absorption. Absorption of ultraviolet and visible light may be measured using a number of different equipment. A monochromator is used to focus the light before it hits the sample and finally the detector in a single beam device. A dual beam instrument is used to compare the intensity of the beam that passes through the sample cell with the intensity of the beam that does not.

A fast spectrophotometer uses detectors with several channels. To avoid interference from

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background substances in a sample, researchers may use differential spectroscopy to correct for their presence. The sample is illuminated by two monochromatic beams of light, one at each of two distinct wavelengths, in a dual wavelength Spectrophotometer. One wavelength is typical of the medication, while the other is chosen with care to achieve absorbance equal to that which is expected at the analytical wavelength. As so, the second beam of light functions similarly to the reference cell in a traditional difference Spectrophotometer. First, second, and higher order derivatives of a sample's absorbance as a function of wavelength are calculated using derivative spectroscopy. This technique has found widespread use in forensics and biomedical research. Most modern pharmacological analyses involve separating the substance in question and then measuring its UV-Vis absorption. As a detector in HPLC and UV is often employed to authenticate the authenticity of other analytical data.

A range of natural items with potential as medicines are examined using spectroscopic methodology, e.g.lobeline (249nm), reserpine (268), morphine (286 nm), cardioactive glycosides (217 nm), colchicines (360nm), vanilline (301 nm), etc. The phytoconstituents analyzed in Visible range are, morphine (442 nm by nitroso-reaction), anthraquinone (505 nm after treatment with alkali), Cardioactive glycosides (590nm) by keller-kiliani reaction), cyanogenetic glycosides (630 nm by pyridine pyrazolon colour reaction), etc.

Instrumentation:

Through a sample, light with a certain intensity and frequency range may pass and % transmittance measured using a spectrophotometer. To do this, it measures the ratio between the incident and transmitted light intensities. Light sources, monochromators, detectors, amplifiers, and recording devices make up the contemporary ultra-violet-visible spectrometer. Red radiations, or radiations with a wavelength of 375 nm, are abundant in tungsten filament lamps. While the area below is illuminated by the deuterium discharge lamp. The deuterium discharge source weakens when its wavelength is greater than 360 nm. Overall, the UV-VIS spectrum agrees that the single source is enough. The typical range of a spectrometer is 220-800 nm. Most modern devices can detect wavelengths as short as 185 nm. The spectroscopic approach being used is useless below 200 nm. (Inaccessible zone) due to the high oxygen absorption below 200 nm. Total path length evacuation is used for sub-200 nm absorption studies. Vacuum ultraviolet refers to the spectrum of wavelengths less than 200 nm. By flushing the sensor with nitrogen, which has low-wavelength absorption down to 150 nm, the usable range of the device may be expanded. Double-beam devices are the norm for spectrophotometers. The main light source is split down the middle into two equal beams. A revolving prism is used to spread the incoming radiation out before it is split into two beams. A prism is used to divide the wavelengths of a light source, and then slits are used to choose the desired wavelengths such that, when the prism is rotated, a progressively longer and longer wavelength of light is recorded. The chosen beam is monochromatic and is split into two equal-power beams. A dispersion grating may also be used to convert UV-VIS photons into a monochromatic beam. Due to the low dispersion of a single beam or grating, extremely narrow band widths cannot be isolated or collimated. So, following the first dispersion, the light travels via the second dispersion and finally exits through the exit slit. The second dispersion improves upon the first because the emerging light has a wider band width and nearly monochromatic light leaves through the exit aperture. Additionally, there is a large reduction in ambient light.The sample solution is passed through one of the monochromatic laser beams, while the reference solvent is sent through the other. Cells composed of material that is transparent in the research area might hold the solvent and solution of the sample. Since glass has a high absorption in the ultraviolet range, it can't be utilized. It's possible to employ silica cells. Never touch the optical surfaces of these, and always keep them in a safe place. In addition, quartz cells perform well in this role. In the range of visible light, glass performs well. Double beam spectrophotometer is the name given to this particular kind of spectrometer. Always measured together with the absorbance of the pure solvent is the absorbance of the solution. In most cases, diluted solutions are scanned as the sample. The solution is made up to 100 ml volume by dissolving 1 milligramme of the substance under study (molecular weight 100-200) in a suitable solvent. A silica cell is used to sample some of the fluid. It's recommended that the solution in the cell be 1cm thick. The absorptivity is occasionally written as E1%1cm when the make-up of the absorbing substance is unknown. An identical cell (the reference cell) is used to collect pure solvent. The spectrometer's monochromatic beams are shone on these cells at the same intensity. After both the sample cell and the reference cell have been

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illuminated, the transmitted beam intensities are compared over the instrument's whole spectral range. The spectrometer uses an electronic subtraction process to determine the difference between the reference beam's absorption and the solution's absorption. As a result, the effects of light absorption by the solvent are reduced to a minimum. This allows for a direct measurement of the compound's absorbance or transmittance. Absorbance intensity is shown on a graph against the matching wavelength. Typically, a graph of absorbance A (log10 Io/I) vs wavelength (abscissa) is used to represent a spectrum. Maximum Extinction Coefficient vs Wavelength is a common representation of this figure. As light is absorbed by the sample, its intensity decreases.

This result in a strong beam from the reference cell being sent to P1 & a weak laser being transmitted to P2 from the sample cell. As a result, alternating currents are generated by the photoelectric cells and sent to the electrical amplifier. The pen recorder is driven by a tiny servomotor that is attached to the amplifier. As a result, the absoption bands are immediately logged. To make sure that the photoelectric cell receives light from both the sample and the reference beams at the same intensity, the amplifier is coupled to a miniature servomotor that moves an optical wedge in the reference beam. Figure 1 shows the uv-vis spectroscopy schematic diagram.

Applications

Analytes such as biological macromolecules, highly conjugated chemical compounds, and transition metal ions can all be quantified are common place applications of UV/Vis spectroscopy in analytical chemistry. While solutions are the most typical medium for spectroscopic examination, solids and gases may also be examined.

Solutions of transition metal ions can be coloured because d electrons inside metal atoms may be stimulated from one electronic state to another (i.e., absorb visible light). The addition of other species, such as certain anions or ligands, greatly affects the colour of metal ion solutions. The addition of ammonia increases the colour and shifts the wavelength of maximum absorption (max) in a copper sulphate solution, for example. Organic molecules, particularly those with a high degree of conjugation absorb ultraviolet or visible light range. These analyses typically use water for chemicals that are miscible with water or ethanol for those that are miscible with organic solvents. (Some organic solvents may have high UV absorption, and other solvents should not be used for UV spectroscopy due to this possibility. In general, ethanol has a low absorption coefficient. The organic compound's absorption spectra may be modified by the polarity and pH of the solvent used. Charge transfer complexes frequently produce hues; however the hues are frequently too intense to be used for quantitative analysis, as seen by the tyrosine's absorption maxima and molar extinction coefficient, which increase with increasing pH from 6 to 13 or decreasing solvent polarity.

An analyte's presence elicits a reaction that is believed to scale linearly with its concentration. The practise of comparing an instrument's response to an unknown analyte with its response to a standard is essentially analogous to the use of calibration curves. The response factor is the response (e.g., peak height) for a given concentration.

Peaks of absorption are helpful in identifying functional groups inside a molecule since their wavelengths are associated with the kinds of bonds present. Conjugated organic substances like dienes and ketones, for instance may have their maximum UV/Vis absorption wavelength, or max, predicted using the Woodward-Fieser criteria, which are based on empirical data. However, the spectrum is not sufficient as a stand-alone test for any particular sample. The absorption spectrum may be affected by factors such as the kind of solvent used, the solution's pH, the temperature, the amount of electrolytes present, as well as the existence of any disruptive substances. Additionally, experimental variables like the spectrophotometer's slit width may have an impact on the spectrum. These factors must be managed or taken into consideration while analysing using UV/Vis spectroscopy.

UV-Vis spectroscopy is also used in the semiconductor sector to measure the optical properties and thickness of thin layers on a wafer. When analysing data from UV-Vis spectrometers, the Forouhi-Bloomer dispersion equations can be used to determine a film's Index of Refraction (n) and Extinction Coefficient (k) for the spectral range that was recorded.

2. Infra Red Spectroscopy[1]

Infrared (IR) spectroscopy focuses on radiant energy that is reflected, absorbed, or transmitted in the 0.8-500 nm region of the electromagnetic spectrum. The frequency, often measured in terms of wave numbers, is the most usual unit of analysis. Near IR (12,500–4,000 cm-1), mid-IR (4,000–8,000

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cm-1), and far-IR (above 16,000 cm-1). In the pharmaceutical industry, infrared refers specifically to the mid-IR band, which is commonly employed for analysis.

A single- or double-beam infrared spectrophotometer may be used. Recent developments in IR spectroscopy have led to the development of the Fourier transform spectrophotometer, which offers several benefits over dispersive devices. They can scan fast, making them useful for capturing spectra when chemicals are eluted in gas or liquid chromatography. Since IR spectra can be obtained with relative ease from a wide variety of sample types—including insoluble solids, polymers, solutions, and gases—For the identification of medications, polymorphic modification, excipients, and raw ingredients in pharmaceutical products, it is widely utilised. It is very likely that two samples have the same chemical structure if their IR spectra are identical (super-imposable). The discovery of functional groups in bio-molecules by IR spectrometric analysis is a powerful technique for understanding their structures. Antibiotics, alkaloids, quinine and strychnine (6.2µ & 6.06µ), steroidal sapogenin (11.11µ), and other substances can all be quantitatively analysed. The schematic diagram of FT-IR is present in fig 2.

3. Nuclear Magnetic Resonance Spectroscopy [1,2]

It deals with the description of substances held in a magnetic field while they absorb radio frequency radiation. Minimal resonance signals are often used to generate NMR spectra. Deuterated versions of common solvents like water, chloroform, acetone, etc. are now readily accessible. For the most part, nuclear magnetic resonance (NMR) has proven the most useful technique of identifying stereochemistry and configuration for molecule. It may reveal the location of protons inside a complicated molecule. It is the gold standard for qualitative analysis in the pharmaceutical industry. It is an absolute approach in quantitative analysis since it does not need a reference standard of the substance under study. Due to its convenience, speed, and specificity, NMR has found several uses in the analysis of contaminants and trace compounds in mixtures. NMR can also be merged with mass spectrometer.

Internal Standards [2]

Internal standards are frequently derived from the following references:

1. TMS, or tetramethyl silane: In NMR spectroscopy, it is frequently employed as an internal standard to determine the locations of 1H, 13C, and 29Si. The reasons for this are as follows:

- It is chemically inert and miscible equivalent.
- Its twelve protons are magnetically comparable to one another.
- It is quite flammable and is simple to remove in order to obtain the sample back.
- It does not interact with the sample's molecules in intermolecular interactions.

Most organic compounds' proton-induced absorptions are distant from their resonance sites. In NMR spectroscopy, it is frequently employed as an internal standard to determine the locations of 1H, 13C, and 29Si.

2. Trimethylsilyl-3-propane sulphonate sodium salt: The substance is water soluble. It serves as an internal benchmark when running water-soluble material PMR spectra in a deuterium oxide (D_2O) solution.

Solvents used [2]

Proton-free solvents or materials that show no absorption in an NMR spectrum should be used. In addition, the solvent must be able to dissolve at least 10% of the material of interest. Hexachloroacetone (CCl3)2 CO, deuterochloroform (CDCl3), carbon tetrachloride (CCl4), carbon disulfide (CS2), etc. are all extensively used solvents in nuclear magnetic resonance (NMR) spectroscopy. The polarity of these solvents varies widely. It makes sense that a molecule's NMR spectra obtained in one solvent might change slightly from those obtained in another solvent with a different polarity. That's why it's crucial to label NMR spectra with the solvent it was acquired in. The strength of hydrogen bonding in this method also affects the value of. Due to the nature of hydrogen bonding, which involves the transfer of electron clouds from a hydrogen atom to a nearby electronegative atom (O, N, S, etc.), hydrogen has a net deshielding impact. Its signal emerges at larger value when the concentration of -OH, -NH compounds is high (strong intermolecular hydrogen bonding), since the proton is deshielded at a higher concentration. The strength of hydrogen bonds decreases as temperature rises. Therefore, at elevated temperatures, the NMR signal emerges at a lower value. Resonance-stabilized dimers of carboxylic acids occur through hydrogen bonding. As a result, the signal for carboxylic -OH in carboxylic acids appears between 10.5 and 12. The

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value of for a molecule exhibiting intramolecular hydrogen bonding is unaffected by the concentration of water in the solution. Since trifluoroacetic acid's proton absorbs at a rather weak field (δ =11.2), it is a good solvent that does not significantly alter the majority of spectra. Particularly helpful for amine NMR spectra. Substituted aliphatic and aromatic ammonium ions have proton absorption edges between 0.5 and 2.9 (aromatic at low field). NMR spectroscopy may make use of dimethyl sulfoxide (DMSO), a highly polar solvent. The solvents employed in this technique often exhibit the following properties.

- It has to be chemically and magnetically neutral.
- It is important that the solvent (ii) does not include any hydrogen atoms, and effectively dissolves the sample.
- The tau values obtained for the different samples may also need to be adjusted by an amount of 0.5 ppm or more if the solvent used was pyridine, benzene, dioxane, trichloro aceto-nitrile, acetonitrile, or dimethyl formamide.
- NMR spectra of solvents that have been entirely deuterated have been discovered to have one or more peaks. It's because the solvent has some trace contaminants.

^{1}HNMR [2]

Principle: All nuclei with an odd number of protons spin on their own axis. An external magnetic field (Ho) causes the nucleus to spin on its own axis, producing a magnetic moment and setting off a chain reaction that leads to a precessional orbit with a certain frequency. The term "ground state" or "parallel orientation" is used to describe this condition. In this configuration, the nucleon spin-induced magnetic field is parallel to the applied magnetic field. Absorption of energy takes place and an NMR signal is recoded when radiofrequency energy is delivered and delivered frequency = Precessional frequency. The ensuing spin reversal occurs when a nucleus absorbs enough energy to change from its ground state to its excited state or antiparallel orientation causes the nucleus's magnetic field to be aligned anti-parallel to the external magnetic field. When the radiowaves are turned off, the nucleus reverts to its natural, parallel condition. When the magnetic field intensity is increased, the precessional frequency rises but there is no change from the ground state to the excited state. There are only average spins and no two-spin states outside of a magnetic field. Therefore, it is impossible to absorb radio waves. In order to produce NMR spectra, a magnetic field and radio waves must be applied.

Instrumentation

A nuclear magnetic resonance spectrophotometer is composed of a magnet, radio waves, a detector, and an amplifier. The detecting apparatus may measure the energy transfer from the radio-frequency beam to the nucleus. o conduct the analysis, a glass tube containing the sample is placed between the two poles of a magnet. The sample is subjected to radiation from a radio transmitter (υ = 60 mega cycles per second). You may achieve this by connecting a coil around the sample tube and supplying it with energy from a radio-frequency source. When the sample's nuclei have the same energy as the source, the resulting signal is known as a resonance. From the nuclei, energy is transferred to the detection coil. After signal amplification, the detector's output may be recorded on a chart strip. Since protons in a molecule occupy widely varying electronic states, they are unable to resonate at a uniform frequency of 60 mega cycles per second. Putting a tiny electromagnet on primary magnet face pole allows field strength to be adjusted while keeping the radio-frequency source stable at the specified frequency. The overall field strength may be raised by increasing the current passing through these electromagnets. Each proton's precessional frequency grows according to the intensity of the field, eventually resonating alongwith RF source. Resonance occurs when H or H group analogous causes a peak in the detector's signal, which is shown graphically on paper. The NMR spectrum displays a series of peaks that are directly proportional to the magnitude of the external magnetic field. Each crest represents a distinct group of protons. Fig.3 shows a schematic diagram of a nuclear magnetic resonance (NMR) spectrometer.

^{13}CNMR spectroscopy [2]

The use of 13C-nuclear magnetic resonance is a cutting-edge method. The 1H NMR and 13C NMR spectra look and were recorded differently, and there are significant variances between the two. For 12C, the value of the spin quantum number I is 0. Therefore, it does not exhibit magnetic properties and does not produce an NMR signal. The nuclear magnetic resonance of 13C may be detected under 23500 Gauss magnetic strength, a frequency of 25.2 mega cycles per second, since its spin quantum number is equal to

IJPRA Journal
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half a. One thing to keep in mind is that 1H NMR may be seen at 100 mega cycles per second when the same magnetic field is used. C-13 isotope has low 1.11 percent abundance in nature. The already low abundance further dampens the absorbance's sensitivity. While the element's scarcity had hampered the development of 13C NMR, we now see that nature made a fortuitous selection. In fact, if nuclei were more abundant, the immense complexity of spectra resulting from heteronuclear and homonuclear spin-spin coupling would have prevented either proton or 13C NMR from ever becoming a significant tool for the organic chemist. The typical technique for getting a 1H NMR spectrum involves sweeping the excitation frequency or the field over the spectrum of nuclear precession frequencies. The fact that, at any given instant, only one line can be visible demonstrates the inefficiency of this approach. This issue manifests itself in the study of 13C, whose naturally thin lines span a large absorption range. Therefore, it is beneficial to excte the whole frequency spectrum all at once. This is accomplished by simultaneously activating all of the relevant resonances with a powerful radio-frequency pulse over a wide frequency range. Following the pulse, the nuclei will freely precess at frequencies that correspond to the local chemistry. In addition to the proton that is immediately attached, each 13C resonance in an organic molecule is spin linked to the proton that is two to four bonds away. When the 13C absorbs a proton that is immediately linked to it, the coupling constant is more than 125 cps (JC-H >125 Hz). The coupling constant is close to 20 cps when the coupling proton is between two and four bonds away. Therefore, multiples with unresolved long range couplings may be seen in 13C NMR spectra. Each signal looks as a hump on the graph. Because there are so many one-bond C-H couplings, the overlapping of multiplets further complicates the spectrum.

The simplification of 13C NMR spectra was made possible in large part by the development of a proton decoupling method. This method relies on a narrow excitation band centred on a single 1H decoupling frequency. The separation of the proton noise is called that. Pseudorandom noise is used to modify this single frequency, resulting in effective excitations throughout a specified frequency range. In order to detect all protons in a given sample, the band width may be made sufficiently large.

4. Mass spectrometry[1]

The primary goals of mass spectroscopy are the ionisation of atoms and molecules by an accelerating electron beam, the subsequent fragmentation of these atoms and molecules. Original molecule's structure may be conjectured based on the fragmentation pattern. The primary use of mass spectrometry is in the measurement of molecular weights. Mass spectrometry is the most reliable approach for identifying medication components, whether used alone or in conjunction with chromatographic separation. Fig.3 shows a schematic diagram of a Mass spectrometry.

Instrumentation [2]

The following components make up the apparatus required to generate spectrum compound:

a. As of ion source, ionising the material being studied is the first and perhaps most crucial step in acquiring the mass spectrum. Ionisation potential is the least amount of energy needed to turn an atom or molecule into an ion. Mass spectrometers typically employ electron bombardment to create ions for analysis. An electrically heated tungsten filament is the source of the blasting electrons. The source generates a few milligrammes of the chemical as a vapour at a pressure of 106 mm. A small opening in the ion chamber lets the vapours in. A tungsten filament's electron stream smashes into it here. The energy of the bombarding electrons is around 70 e V. Molecules undergo electron loss during bombardment, resulting in the formation of a parent ion radical.Typically, 10 eV is needed to lop off one electron from a molecule's neutral parent. There is no ionisation or fragmentation of the parent ion at this energy level. However, if the bombarding electron's energy is close to 70 eV, more energy is expended in the fragmentation of the parent ion. In this process, ions known as fragments or daughter ions are created. In a mass spectrometer, only positive ions (fragments) with either an even or odd number of electrons are detected, whereas neutral molecules and radicals are not.

b. The positively charged ions (parent or fragment ions) generated in the ion chamber are accelerated by an acceleration potential in step b of the mass analyzer. The mass analyzer receives these ions at this point. In this case, the m/z ratio serves as the criterion for classifying the fragment ions. A slit B guides the positive ions to their destination. Between points A and B, a repulsive potential is applied. An acceleration potential, on the order of 8kV, is applied across slit C to speed up the ions.

IJPRA Journal

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Between the plates B and C, an acceleration potential is applied. At fast speed, positive ions go through the mass spectrometer's whole analyser and are then sorted based on their m/z values.

c. Detector of Ions (c) Electrical or optical detection and measurement of the ions that have been separated by the analyzer. Ions are individually collected when they fall through the collecting slit. Going higher on the scale means scanning more of the spectrum. A direct current amplifier is used to boost the ion currents. In order to capture the spectrum, a rapid-scanning oscillograph is used. Three to five separate peaks are recorded using galvanometers of varying sensitivity to create this sort of recording.

Applications

a. Nitrogen rule, peak matching, compound fragmentation pattern, and % isotope abundance may all be used to help elucidate the structures of organic compounds. b. Functional group assignment

b. Impurity Detection: Extra peaks, peaks with a higher mass than the chemical itself, and a distinctive fragmentation pattern all indicate the presence of unwanted substances.

c. Quantitative analysis: peak strength is proportional to fragment abundance. Sample size is quantified by comparison to the reference medication.

d. Studies of drug metabolism: The drug's metabolism may be determined by comparing the mass spectra of the metabolite and the pure drug.

e. Uses in the Clinic, the Lab, and the Courtroom. The mass of even trace amounts of the medication allows its identification using mass spectrometry.

5. HPLC [1]

It is a separation technique that employs a liquid medium and a packed column. It is the packing material; it may be deposited as either a solid or a liquid on top of the stationary phase's inert material. Elution is accomplished using a liquid mobile phase. With either autocratic elution or gradient elution, the mobile phase is pushed through the column at high pressure in high-performance liquid chromatography (HPLC).For monitoring the purity of therapeutic ingredients, it has evolved into the most flexible, secure, trustworthy, rapid, and sensitive chromatographic technique. High-performance liquid chromatography can be used to identify a variety of medications, including morphine, codeine, papaverine, emetin, antibiotics, ergot alkaloid, steroids, cardiac glycoside, capsaicin, sennosides, vitamins, & constituents of rhubarb. For the analysis of digoxin and gitoxin, RP column, 65% methanol is used. Many pharmaceutical firms are using HPLC as a standard procedure for the testing of novel medications and as a replacement for more cumbersome tests for already available pharmaceuticals. It is also commonly used in analyses of drug concentrations in blood and other body fluids.

Derivatisation in HPLC is undertaken primarily to increase the sensitivity of detection for a given compound. A compound can be derivatised before or after the column separation. Precolumn derivatisation is most widely used because it allows the greatest flexibility in the choice of the reactions to be used.

A number of commercial HPLC instruments are available with various modification and advancements. In micro bore chromatography; the column used is very narrow (1mm or less). Flow rate of the mobile phase is also very less (100 µl min). This method is useful for regular analysis of drugs in the pharmaceutical industry and it saves the amount of solvents used as mobile phase. In supercritical fluid chromatography, the mobile phase is held at conditions of temperature and pressure such that it has its viscosity and diffusibility midway between gas and liquid form. The advantage of supercritical fluid chromatography is high column efficiency and short time taken for the analysis.

6. Gas Liquid Chromatography(GLC) [1]

In gas chromatography, a gas stream is passed through a stationary phase in order to separate volatile compounds. GLC relies on the sample being separated into liquid and gas phases as the liquid film is spread over an inert material. Since a variety of liquid phases may be used up to 450℃, the most flexible and discerning type of gas chromatography is GLC. The effectiveness of the GLC system will depend on the carrier gas you choose. Nitrogen or helium, which strike a balance between inertness, efficiency, and running cost, are the most often, used carrier gases.

Examples of the kind of issues that GLC is accustomed to in pharmaceutical analysis include the assay of starting materials and drug substances, quantification of drug in formulation, and assay of impurities and/or solvents in the raw material or in drug substances. GLC has a wide range of important applications, including the analysis of several, plant acids, volatile oils, conium, tobacco, opium, belladonna alkaloids, steroidal constituents,

cannabis resins, aglycon, cardioactive glycosides, sugars, and amino acids, etc.

Due to interactions between the functional groups and the stationary phase, many medicines with carboxylic acid or primary amine functional groups produce poorly tailed peaks. Pyrolysis gas chromatography (GC), photolysis gas chromatography (GC), and derivatization are only a few of the specialised methods that have been developed to deal with these chemicals. Pyrolysis GC involves rapidly heating an analytical sample to high temperatures, shattering it thermally, and then sweeping the resulting fragments onto a gas chromatograph.

Derivatization allows for the structural modification of a sample, the most common of which is silation. Alcohols, amines, carboxylic acids, and thiols are all examples of reactive functional groups that may be transformed into more stable compounds such as ethers, silylamines, esters, and thioesters. Trimethylchlorosilane, hexamethyldisilazone, and N, O-bis (trimethylacetamide) are some of the most frequently utilised derivatizing compounds.

7. Liquid chromatography-Mass spectrometry [3-10]

It is an analytical strategy that combines the physical separation capabilities of high-performance liquid chromatography with the mass analysis capabilities of mass spectrometry. Because of complementary nature in chromatography and MS, coupled chromatography-mass spectrometry systems have been widely used in the field of chemical analysis. The spectrum information provided by mass spectrometry may be used in conjunction with the results of liquid chromatography to positively identify (or verify the suspected identification of) each component extracted from a mixture. The necessity for exhaustive chromatographic separation is eliminated by mass spectrometry's sensitive and selective detection. LC-MS is a good choice for metabolomics due to its comprehensiveness over a large range of chemicals. This tandam approach can be used to examine biochemical, carbon containing, and non carbon containing chemicals that are frequently present in environmental and biological complex samples. Therefore, LC-MS may be used in several industries, such as biotechnology, environmental monitoring, the food industry, and the pharmaceutical, agrochemical, and personal care product sectors. LC/MS/MS utilized for therapeutic settings since the early 2000s. In order to transfer the separated components from the LC column to the MS ion source, an LC-MS system requires both an LC column and a mass spectrometer. Due to their inherent differences, the interaction between LC and MS devices is critical. Even though the liquid mobile phase in LC devices is under pressure, most MS analyzers work in a vacuum. This makes it impossible to quickly move the eluate from the LC column to the MS source. The interface, a mechanically straightforward component of the LC-MS system, preserves the chemical identity of the chromatography products while enabling maximum analyte transfer and removing a sizable portion of the mobile phase used in LC. The ionising capabilities and hoover settings of the MS system must be preserved via the interface. Atmospheric pressure ionisation (API) techniques are widely utilised at present in Liquid Chromatography-Mass Spectrometry interfaces, with electrospray ionisation, atmospheric pressure chemical ionisation, and atmospheric pressure photoionization being the most popular.

Liquid Chromatography (LC)

The chemicals in a liquid mixture are separated physically by being split up between two immiscible phases (the stationary phase and the mobile phase). The most popular of these is the reverse phase mode of partition chromatography, which uses a nonpolar stationary phase and a polar mobile phase. The stationary matrix is created by covering the surface and interior of porous silica particles with long-chain alkyl groups like n-octadecyl or C18. The mobile phase is typically composed of aqueous solutions of polar solvents. 20 µl of the target sample are normally added to the mobile phase stream produced by a high pressure pump in high-pressure liquid chromatography. The mobile phase that is carrying the analyte travels through the stationary phase bed along a preset path. The separation of mixture components is dictated by their chemical affinities for the mobile and stationary phases. The repetitive sorption and desorption of the liquid at the fixed bed leads to isolation. Repeatable chromatography experiments are made possible by using high pressure to drive the liquid solvent down a packed column constructed of the stationary phase at a consistent flow rate. Due to the partitioning between the mobile and stationary phases, the compounds in the sample will leave the column at various periods. The column is constructed to withstand the high pressure of the liquid because of its significance to the LC system. Standard column of liquid chromatography range in length from 100 mm to 300 mm and have

an outside diameter of 6.4 mm (1/4 inch) and an inner diameter of 3.0 mm to 4.6 mm. Shorter chromatography columns (30-50 mm) with packing particles of 3-5 µm diameter are appropriate in liquid chromatography-mass spectrometry uses. Along with the conventional design, there are additional varieties of liquid chromatography columns, such as narrow bore, microbore, microcapillary, and nano. These columns can process liquid flows at rates lower than 1 millilitr/minute, offer better separation, and have smaller internal diameters. To increase peak resolution and isolation effectiveness, ultra performance liquid chromatography may be employed instead of conventional HPLC. This liquid chromatography variant uses columns with smaller silica particles and operating pressures ranging from three lakh ten thousands to seven lakh seventy five torr. Mass charge ratio for ion may be calculated using mass spectrometry (MS), an analytical technique. In order to measure the m/z of an analyte, all mass spectrometers use electric or magnetic fields to control the mobility of ions produced by the analyte. The ion source, mass analyzer, detector, data, and vacuum systems make up a mass spectrometer. A mass spectrometer's ion source is where electron beams, photon beams (UV light), laser beams, or corona discharge are utilised to ionise a sample's constituent parts. Ions in a liquid solution are moved to the gas phase by the ion source when electrospray ionisation is used. The ion source transforms and disperses the neutral sample molecules into gas-phase ions before sending them to the mass spectrometer. The detector tracks and amplifies the ion current for each mass-resolved ion, and the mass analyzer employs electric and magnetic forces to separate them. The information system gathers, evaluates, stores, and displays data on a computer to produce a mass spectrum that is easily recognisable to the naked eye.

The mass spectrum may be used to make educated guesses about the mass, elemental, and isotopic composition, or chemical structure of the analytes in a sample. In order to conduct an MS experiment, a vacuum of 1.33 x 10^{-2} to 1.33 x 10^{-6} pascal is required for the gas phase. For MS to become a useful tool in the identification and quantification of organic molecules like peptides, Equipment that makes it easier to transfer materials from a higher pressure and condensed into a vacuum system has to be developed. Mass spectrometry is currently frequently used in analytical labs that look into the physical, chemical, or biological properties of a variety of components. There are numerous types of mass analyzers used in liquid chromatography-mass spectrometry systems, including quadrupole, time of flight, ion trap, and hybrid quadrupole time of flight models.

Ionization by electrospray

Fenn and colleagues developed the ESI interface for LC-MS devices in 1988.Metabolites, xenobiotics, peptides, nucleotides, and polysaccharides are all examples of moderately to highly polar compounds that may be analysed using this ion source/interface. The liquid eluate coming out of the LC column is directed into a metal capillary maintained at 3 to 5 kV and nebulized by a high-velocity coaxial flow of gas at the tip of the capillary in order to produce a tiny spray of charged droplets in front of the entry to the vacuum chamber. In order to avoid buffers and salts from contaminating the vacuum system, this capillary is frequently positioned perpendicularly at the input of the MS system with a counter-current of dry nitrogen in front of the entry through which ions are steered by the electric field. Some sources combine a hot gas stream with the spray plume in front of the vacuum entry to increase ion emission, which causes the droplets to evaporate quickly. In some other sources, droplets are evaporated and ions are emitted by passing them through a heated capillary tube before entering a vacuum. These improved droplet evaporation techniques have made it possible to achieve efficient ionisation and great sensitivity at liquid flow rates as low as 1-2 ml/min. Instead of the more traditional practise of employing 1 to 3 mm microbore columns and lower flow rates of 50 to 200 l/min for optimal performance, the higher column capacity of bigger bore columns can be utilised advantageously with ESI LC-MS systems. By reversing the polarity, both +ve & –ve charge ions produced, and separate spectra for the positive and negative modes can be quickly acquired during the same LC run. The bulk of the ions produced by the ESI source are singly charged, whereas the majority of the ions produced by the big molecules (MW 1500-2000) are multiply charged.

Atmospheric pressure Chemical Ionization (APCI)

Horning and his colleagues in the early 1970s laid the groundwork for the APCI interface used with LC-MS but the economic benefit didn't appear until the early 1990s. Not soluble in water, thermostable, neutral compounds are ideal candidates for study with the APCI ion source/interface. When utilising ESI, these

compounds do not ionise reliably. Mobile phase streams with buffering agents are no problem for APCI. Pushing liquid through a capillary causes nebulization and corona liberation to take place at the tip. The ion source first causes chemical ionisation of the mobile phase solvent and the nearby ionising gas. These ions then combine with the analyte and impart their electric charge. Ion-focusing lenses or tiny orifice skimmers direct the sample ions as they pass through. Once the ions have entered the high vacuum field, their masses will be determined. Single-charged ions may be generated at this interface, and it has both positive and negative charge operating modes. The APCI ion source can be directly linked to conventional 4.6 mm ID columns, and the flow rate can be changed from 500 to 2000 µl/min.

APPI

In 2000, both Bruins and Syage developed the Atmospheric pressure photo ionisation interface for LC-MS.For the study of neutral substances that cannot be ionised using ESI, APPI provides an additional ion source/interface for LC-MS. This interface, like the APCI ion source, generates ionisation using photons from a discharge lamp rather than a corona discharge. The direct-APPI mode produces unpaired molecular ions of the analyte by absorbing a photon and ejecting an electron. The dopant-APPI mode adds an easily ionizable material to the mobile phase or nebulizing gas to enhance the charge-exchange interaction between the dopant molecular ion and the analyte. On its journey to the mass analyzer, where it will be studied, the ionised material will then pass through minuscule orifice skimmers.

Applications

Due to the ability of liquid chromatography to separate complex and delicate natural mixtures, whose chemical composition must be meticulously established, the connection of Mass Spectrometry with Liquid Chromatography systems is interesting. In addition, LC-MS might be helpful when looking into volatile explosive residue. The polar and thermally labile nature of more than 85% of natural chemical components makes LC-MS one of the most used methods for chemical analysis today. GC-mass spectrometry on other hand, can handle these compounds.

Pharmacokinetics

In addition to its widespread use in bioanalysis, LC-MS also plays an important role in the study of drug pharmacokinetics. The rate at which a medication is eliminated from tissues and the liver may be estimated with the use of pharmacokinetic research. Because they are more sensitive and specific than UV detectors, which are frequently used in conjunction with HPLC systems, MS analyzers are advantageous in these investigations. It is advantageous to employ tandem MS-MS because the detector can be configured to only fragment specific ions. The sum of the components of the molecule that the user selects represents the entire amount. Liquid chromatography may be relatively quiet in comparison to LC-MS, which does not suffer from interferences or ion suppression.

Proteomics/metabolomics

In proteomics, LC-MS is used as a method for separating and identifying individual molecules within a complicated sample. Developing proteomics from scratch Standard procedures in the LC-MS approach include protease digestion and denaturation; trypsin is frequently used as the protease, urea is frequently used to denature the tertiary structure, and iodoacetamide is frequently used to alter the cysteine residues. Following digestion, LC-MS is used to perform peptide mass fingerprinting or peptide sequence creation by LC-MS/MS. When doing a proteomic study of complex materials, LC-MS/MS is frequently employed to prevent peptide mass overspreading, even with a high-resolution MS. Modern LC-MS/MS systems are suited for the analysis of complex biological materials since they can recognise over a thousand proteins. Isolating the material by SDS-PAGE gel or HPLC-SCX is required for such a precise protein measurement, nevertheless. Peptide biomarker searches have traditionally made use of LC-MS/MS. LC-MS has proven to be one of the most popular methods for the global metabolite profiling of biological tissue. LC-MS is also used for the profiling of secondary metabolites in plants and the investigation of natural medicinal products. To this end; MS-based methods are useful for learning more about the complicated biological samples' vast range of components. While LC-MS was utilized in metabolomics of plant, it is limited to identifying and approximating the most abundant metabolites. LC-MS has proven important in this endeavour. The discovery of many oligosaccharides, amino acids, sugar nucleotides, and broadly polar metabolites from the phloem tissues of Cucurbita maxima represented the first application of LC-MS in plant metabolomics.

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Drug Development

Due to its speed and accuracy, LC-MS is often used in the pharmaceutical industry throughout the drug development process. These characteristics expedite the process of developing, testing, and verifying recognition, all the while drawing from a wide pool of potentially useful goods. Lipidomics, Bioaffinity screening, Identification of impuriry are just some of the many ways that LC-MS aids in drug development.

8. Gas Chromatography-Mass spectrometry [11-16]

GC-MS is a technique that combines mass spectrometry and gas chromatography to identify various substances inside a sample. Due to the high temperatures of 300 ˚C employed in the GC-MS injection port, thermal degradation of injected molecules may cause the identification of degradation products rather than the complete molecules of interest.

Instrumentation

The two fundamental parts of the GC-MS are the gas chromatograph and the mass spectrometer.The capacity of the gas chromatograph to separate molecules in a capillary column depends on dimension and phase characteristics. Due to differences in their chemical makeup and affinities for the stationary phase, molecules in a sample will become more separated as it travels down the column. Since the molecules are retained by the column and elute from the column at different times, the mass spectrometer can collect, ionise, accelerate, deflect, and identify each of the ionised molecules separately. The molecule is ionised, and the detection is performed utilizing the mass-to-charge ratio of the resultant fragments. These two aspects work far better together than they do separately for subtle chemical identification. The identification of a chemical cannot be made with absolute certainty using only gas chromatography and mass spectrometry.When employing the mass spectrometry method, two or more molecules will often co-elute since conventional gas chromatography detectors are unable to distinguish between molecules that appear to take the same amount of time to pass along the column. In a mass spectrometer, the ionised fragments (or "peaks") of two different molecules may sometimes seem quite similar to one another. Combining the two processes reduces the possibility of error because it is extremely unlikely that two different molecules would behave similarly in a mass spectrometer and a gas chromatograph. As a result, determining the mass spectrum appears at a certain retention period in a GC-MS investigation may be achieved to increase confidence that the analyte of interest is present in the sample.

Purging –Trap Gas chromatography-Mass spectrometry

Volatile samples may be introduced instument. Purging or sparging is the process of extracting target analytes from a mixture of the substance and water in an airtight chamber using inert gas, such as N_2 gas. The volatile substances enter the headspace above the water and are pushed out of the chamber by the pressure differential created by the purge gas. Trap is a hot line onto which the volatile chemicals are pulled. The trap is an adsorbent column that transforms the substance it contains back into a liquid at normal temperature. Once the trap has been heated, the sample components are introduced to the GC-MS column using the volatiles interface, a split inlet system. The best candidates for P&T GC-MS analysis are volatile organic substances and aromatic compounds related to petroleum.

Types of mass spectrometer detectors

Commonly used with GCs is the quadrupole mass spectrometer, sometimes known as the "Mass Selective Detector" (MSD) after its manufacturer, Hewlett-Packard (Agilent). The ion trap mass spectrometer is yet another common kind of detector. A magnetic sector mass spectrometer is another possibility; however, due to its high price and large size, it is not often encountered in high-throughput service labs.

Gas chromatography-Mass spectrometry/ Mass spectrometry

When a second stage of mass fragmentation is added, for as when utilising a second quadrupole instrument, tandem MS (MS/MS) is employed. Tandem MS may be used to measure low quantities of target compounds when there is a significant sample matrix background.The first quadrupole (q1) is connected to a collision cell (q2) and a second quadrupole (q3). Any of the quadrupoles may be employed in a scanning or static mode when conducting an MS/MS research. Different types of analysis include the product ion scan, precursor ion scan, and selective reaction monitoring. Here's an illustration: When q1 is in static mode and q3 is in scanning mode, a product ion results. A specific product ion can be selected

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from the spectrum to act as the precursor ion's ultimate product. The two are crucial to SRM and operate together as a transition. SRM has a very low matrix background and a high degree of specificity. Molecules are ionised in a mass spectrometer using a number of different techniques once they have travelled stationay phase, crossed, entered instrument. The fragmented sample is subsequently detected. Whether full scan or SIM is used has no bearing on the ionisation method used.

Electron ionization

Electron ionisation (EI) is the gold standard for ionisation in its many forms. Molecules are struck by free electrons released by a filament akin to that of a standard light bulb after entering the mass spectrometer. The molecules are fragmented in a predictable and consistent manner due to the bombardment of electrons. . By using this "hard ionisation" method, more low-m/z fragments are produced, and relatively few molecules with a molecular weight of one or fewer are created. Hard ionisation is the utilisation of molecular electron bombardment, according to mass spectrometrists, whereas "soft ionisation" refers to the charging of molecules through contact by outside gas. The electron energy input into the system, which is around 70eV, determines the pattern of molecule fragmentation. Utilising &0eV, produced spectra can be quickly compared to library spectra.

Cold electron ionization

When molecules are cooled before being ionised, the harsh ionisation process of electron ionisation is mitigated, resulting in more informative mass spectra. This process, known as cold electron ionisation (cold-EI), involves the molecules expanding into vacuum through a specially designed supersonic nozzle after leaving the GC column and mixing with extra helium make-up gas. When the analyte molecules collide with the make-up gas at the expanding supersonic jet, less fragmentation is produced by the electrons during ionisation because their internal vibrational (and rotational) energy is reduced. When typical fragmentation pattern is preserved, cold-EI mass spectra may be identified using library search techniques because they are easily differentiated by the presence of a large molecular ion. Increased molecular ions improve the accuracy with which known and new compounds may be identified, intensify the effects of isomer mass spectroscopy, and make it possible to employ isotope abundance analysis to determine an element's molecular formula.

Ionization of chemical

During chemical ionisation, a reagent gas—often methane or ammonia—is supplied into the mass spectrometer. Regardless of whether positive or negative chemical ionisation is employed, this reagent gas reacts with the analyte and electrons to provide a "soft" ionisation of the target molecule. When compared to EI, the amount of molecular fragmentation caused by softer ionisation is much less. Chemical ionisation is useful because it produces a fragment ion with a mass that is relatively close molar mass of substance to be determined. In PCI, the reagent gas and target molecule are frequently combined through a proton exchange. This results in the production of large numbers of the species. The reagent gas in negative chemical ionisation (NCI) modifies the free electrons' interaction with the analyte of interest. Because of this depletion of energy, the fragment is usually abundant.

Analysis

The two most common applications for a mass spectrometer are full scan and selective ion monitoring (SIM). Depending on its configuration, a typical Gas Chromatography-Mass Spectrometry equipment may perform either function independently or simultaneously.

Quantifying a material is the fundamental objective of most instrument analyses. The atomic masses in the resulting spectrum are compared for concentrations to accomplish this. It is possible to do either a comparative or an original analysis. First, a spectrum is compared to a library of other spectra to check whether any other samples in the library have its properties. Because of the wide range of possible visual distortions caused by differences in size, this is best accomplished on a computer. To better connect particular facts, computers may concurrently correlate additional data (such the retention durations obtained by Gas Chromatography). Using deep learning to identify VOCs in unprocessed GC-MS data showed encouraging results.

Peak-to-peak distance is another way of examination. In this method, the highest peak receives the whole value (100%) while the remaining peaks get a fraction of that value (based on their height). We've allocated all values more than 3 percent. Typically, the parent peak will show the complete mass of the unknown molecule. This parent peak's value may be used to adjust a chemical formula to account for the multiple components thought to be present in the compound. The isotope

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pattern in the spectrum can be used to infer the molecular structure and bonding, but it must match the characteristics identified by GC-MS. Presented with a list of the potential sample components the instrument's included software can usually make this assessment automatically. All the "peaks" in a spectrum are taken into account by a "full spectrum" study. In contrast, selective ion monitoring (SIM) focuses on a narrower set of ions that are relevant to a certain molecule. This is done on the premise that a certain group of ions is indicative of a particular molecule at a particular retention period. If the analyst already knows something about the sample or is just interested in a small number of substances, this kind of analysis may be completed quickly and effectively. As less information is gathered about the ions in a particular peak, the sensitivity of a gas chromatographic study increases. As a result, even if SIM analysis enables the identification and evaluation of a lower amount of a chemical, the degree of certainty regarding the identity of that compound is diminished.

Mass spectrometry full sacan

During data collection utilising the complete scan mode, a target range of mass pieces is established and incorporated into the instrument's approach. An example of a typical mass fragment range to track is mass/charge 50 - 400. What one expects to be present in the sample, taking into account the solvent and other potential interferences, is a major factor in determining the appropriate range to utilise. Air (found as m/z 28 owing to nitrogen), carbondioxide (m/z 44), and other potential interferences may be detected if the mass spectrometer is set too low to seek for fragments. In addition, the instrument's sensitivity drops because of the decreased number of scans per second required to recognise a broad variety of mass fragments while using a wider scan range. Unknown substances in a sample may be found with the use of a full scan. When compared to SIM, it generates more data that may be used to confirm or resolve chemicals in a sample. When developing a new instrument method, it is not uncommon to start with a complete scan mode analysis of a test solution to establish retention duration and a mass fragment fingerprint.

Selective ion monitoring

Selective ion monitoring (SIM) is a method in which a mass spectrometer is used to detect and identify certain ion fragments. SIM's benefits include a lower detection limit thanks to the limited number of pieces examined by the device at any one time. In a single second, several scans may be performed. Matrix interferences are often smaller when only pieces of low mass are of interest. To further confirm the likelihood of a favourable result, the ion ratios of the individual mass fragments should be compared to a validated reference standard.

Applications:

Decontamination and environmental

Watch to monitor organic pollution surrounding, gas chromatography-mass spectrometry quickly replacing other methods. As GC-MS technology has become more affordable and dependable, it has become more commonplace in environmental research.

Criminal forensics

Human body particles may be analysed by GC-MS to provide evidence connecting an offender to an offence. For the GC-MS analysis of fire debris, there is a recognised American Society for Testing and Materials (ASTM) standard. GCMS/MS is helpful since very exact results are needed when they are used in court. Canines trained to sniff out illegal narcotics are being replaced by the Police GC-MS, which is now the accepted practise. GC-MS is frequently used in forensic toxicology to find drugs and/or poisons in samples acquired from suspects, victims, or the deceased. Liquid-liquid extraction is frequently used to separate target compounds from blood plasma as part of sample preparation for GC-MS-based drug screening.

Anti-doping analysis Lab for sports

Labs rely heavily on GC-MS analysis of urine samples to detect the presence of banned performance-enhancing substances.

Chemical engineering

For the examination of unknown organic chemical combinations, GC-MS is used. The use of GC-MS to analyse the chemical makeup of bio-oils produced from unprocessed biomass is a crucial use of this technology. In order to identify the continuous phase component in magnetorheological fluid, a smart material, GC-MS is also used.

Food, beverage & perfume analysis

Various constituents of aromatic, few of which occur in drug substance & others of which are created during processing, may be found in foods and drinks. To learn more about these components, such esters, fatty acids, alcohols, aldehydes,

IJPRA Journal

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terpenes, etc., GC-MS is often used. Furthermore, it is used to detect and assess potentially dangerous rotting or adulteration pollutants, which are generally governed by government authorities.

Medicine

Newborn screening tests, particularly those using gas chromatography-mass spectrometry, may now detect inborn errors of metabolism (IEM) in infants. Compounds in urine, no matter how minute their concentration, may be identified by GC-MS. People with metabolic disorders tend to have higher than average levels of these substances. An earlier diagnosis and the start of therapy may improve the prognosis of IEM; hence this method is gaining popularity. A GC-MS-based newborn urine test may now detect over a hundred different inherited metabolic abnormalities.

GC-MS was utilized in activity of metabolism identification in conjunction with isotopic tagging of metabolic components. An IRMS is one kind of mass spectrometer utilized for calculation of a ions number and data return like ratio; most applications rely on 13C as the labelling and IRMS for estimating 13C-12C ratios.

9. Polarimeter [17,18]

The angle of rotation created when linearly polarized light passes through an optically active material is measured by a polarimeter, a scientific tool. Certain chemical compounds are optically active, meaning that when linearly polarized (unidirectional) light passes through them, it rotates either clockwise to the right or counterclockwise to the left. The angle of rotation is the degree of rotation of the light. The rotation's magnitude and direction (clockwise or counterclockwise) provide details about the sample's chiral characteristics, including the relative concentration of its enantiomers.

Principle

Polarimetry can be used to determine the concentration, ratio, and purity of two enantiomers. One characteristic of enantiomers is their ability to spin the linearly polarized light plane. As a result, those substances are referred to as optically active, and their characteristic is known as optical rotation. Electromagnetic waves at the visible light frequency are released by light sources like the sun, Tungsten Halogen, and light bulbs. In relation to their direction of propagation, their electric field oscillates in every conceivable plane. On the other hand, linear-polarized light waves oscillate on parallel planes.

Only the portion of light that oscillates in the polarizer's designated plane can pass through when it comes into contact with it. The plane of polarization is the name given to such plane. Optically active chemicals rotate the plane of polarization. The enantiomer is either dextro-rotatory or levo-rotatory, depending on which way the light rotates.

Enantiomers have additive optical action. The optical activity of various enantiomers increases when they coexist in a single solution. Because they negate their clockwise and counterclockwise optical activities, racemates are optically inert. The concentration of optically active compounds in solution is directly correlated with the optical rotation. Therefore, polarimeters can be used to measure the concentration of enantiomer-pure materials.

When describing a new chemical, polarimeters can also be used to determine the specific rotation when the sample's concentration is known. The optical rotation α at a route length l of 1 dm, a concentration c of 10 g/L, a temperature T (often 20℃), and a light wavelength λ (typically sodium D line at 589.3 nm) is known as the specific rotation, which is a physical property:

$$[\alpha]_{\lambda}^{T} = 100 \text{ X } \alpha / 1 \text{ X C}$$

This indicates the amount that the plane of polarization rotates when a light beam travels through a given number of optically active molecules in a sample. As a result, temperature, concentration, wavelength, path length, and the material under study all affect the optical rotation.

The principles, instrumentation, and applications of the many tools used for organic compound characterization are the main topics of this review paper.

Construction

The polarizer and analyzer are two Nicol prisms that make up the polarimeter. The analyzer can be rotated, but the polarizer is fixed. One way to conceptualize the prisms is as slits S1 and S2. It is possible to think of the light waves as corresponding to the string's waves. The S1 polarizer only permits light waves that travel in a single plane. The light becomes plane polarized as a result. Light waves from the polarizer can travel through the analyzer when it is positioned similarly. The field appears dark when it is turned via the right angle because no waves can pass through it. The analyzer must rotate at the same angle if a glass tube filled with an

optically active solution is positioned between the polarizer and analyzer. This is because light now rotates through the plane of polarization at a specific angle.

Operation

Monochromatic light is passed through the first of two polarizing plates to create a polarized beam, which is how polarimeters measure this. The polarizer is the name of this initial plate. After that, as the beam travels through the sample, it is rotated. A second polarizer, referred to as the analyzer, rotates either manually or automatically after passing through the sample. The angle of rotation, which is equal to the angle θ by which the analyzer was rotated in the former scenario or 90-θ in the later, can be determined when the analyzer is rotated so that all or no light can flow through.

10. Differential scanning calorimetry (DSC) [19-21]

The reference and sample are maintained at nearly the same temperature throughout the experiment; a DSC analysis's temperature program is typically designed so that the sample holder temperature rises linearly with time; the reference sample's heat capacity should be well established over the range of temperatures to be scanned. This thermoanalytical method measures the variation in the quantity of heat needed to raise a sample's and reference's temperature as a function of temperature.

Furthermore, the reference sample needs to be highly pure, stable, and not significantly altered throughout the temperature scan. Metals like indium, tin, bismuth, and lead have historically been used as reference standards; however, new standards, such polyethylene and fatty acids, have been suggested to investigate polymers and organic molecules, respectively.

There are two primary types of DSC: power differential DSC, which measures the difference in power provided to the sample and a reference, and heat-flux DSC, which measures the difference in heat flux between the sample and a reference (also known as multi-cell DSC).

Heat-flux DSC

Heat-flux DSC uses the integration of the ΔTref- curve to calculate changes in heat flow. In this type of experiment, a sample and a reference crucible are set atop a sample holder that has built-in temperature sensors to detect the crucibles' temperatures. This setup is found in an oven with temperature control. The unique characteristic of heat-flux DSC, in contrast to the conventional design, is the use of flat temperature sensors positioned vertically around a flat heater. With this configuration, a compact, lightweight, and low-heat capacity structure can function similarly to a standard DSC oven.

Power differential DSC

The sample and reference crucible are positioned in thermally insulated furnaces for this type of setup, sometimes referred to as power compensating DSC, rather than next to one another in the same furnace as in heat-flux-DSC investigations. After then, both chambers' temperatures are regulated to maintain the same temperature on both sides. The temperature differential between the two crucibles is then not recorded, but rather the electrical power needed to achieve and sustain this state.

11. Thermogravimetric analysis or **thermal gravimetric analysis** (**TGA**)

This method of thermal analysis tracks the mass of a sample as its temperature changes over time. This measurement covers chemical phenomena like chemisorptions, thermal breakdown, and solid-gas reactions (such oxidation or reduction), as well as physical events like phase transitions, absorption, adsorption, and desorption.

Thermogravimetric analyzer [22-32]

A device known as a thermogravimetric analyzer is used to perform thermogravimetric analysis (TGA). As a sample's temperature changes over time, a thermogravimetric analyzer constantly measures mass. In thermogravimetric analysis, mass, temperature, and time are regarded as base measurements from which many other measurements can be derived.

A precision balance with a sample pan inside a furnace with a programmable control temperature makes up a typical thermogravimetric analyzer. The temperature is frequently increased steadily to cause a thermal reaction (or, in some cases, managed for a constant mass loss). In addition to oxidizing/reducing gases, corrosive gases, carburizing gases, ambient air, vacuum, inert gas, and liquid vapors, the thermal reaction can occur in a variety of atmospheres and pressures, including high vacuum, high pressure, constant pressure, and controlled pressure.

A plot of mass or the percentage of starting mass on the y-axis against temperature or time on the x-axis is created using the thermogravimetric

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Volume 11, Issue 1, Jan - Feb 2026, pp:978-999 www.ijprajournal.com ISSN: 2456-4494

data gathered from a thermal reaction. A TGA curve is the name given to this figure, which is frequently smoothed. Plotting the first derivative of the TGA curve (the DTG curve) can reveal inflection spots that are helpful for both differential thermal analysis and in-depth interpretations. Characteristic decomposition patterns can be analyzed using a TGA to characterize materials. This approach is very useful for studying polymeric materials, including thermoplastics, thermosets, elastomers, composites, plastic films, fibers, coatings, paints, and fuels.

Types of TGA

Three varieties of thermogravimetry exist:

• Static thermogravimetry, also known as isothermal thermogravimetry: This method records the sample weight over time at a fixed temperature.

• Quasistatic thermogravimetry: This method involves increasing the sample temperature in successive steps that are separated by isothermal periods. The sample mass stabilizes before the subsequent temperature ramp begins.

• Dynamic thermogravimetry: This method involves heating the sample in a linearly changing environment.

Applications:

Thermal stability

A material's thermal stability can be assessed using TGA. If a species is thermally stable, no mass change will be seen within a desired temperature range. The TGA trace has minimal to no slope when there is negligible mass loss. TGA also provides a material's maximum use temperature. The material will start to deteriorate at higher temperatures.

Polymers are analyzed using TGA. TGA is mostly used to examine the thermal stability of polymers because they typically melt before breaking down. Before 200 ˚C, the majority of polymers melts or breaks down. A class of thermally stable polymers that can withstand temperatures of at least 300℃ in air and 500℃ in inert gases without undergoing structural changes or losing strength can be investigated using TGA.

Oxidation and combustion

The residue left over after a reaction is the most basic way to characterize a material. For instance, a sample might be loaded into a thermogravimetric analyzer under normal conditions to assess a combustion process. By heating the material over its ignition temperature, the thermogravimetric analyzer would induce ion combustion. The residue at the curve's end would be seen on the resulting TGA curve plotted with the y-axis as a percentage of beginning mass. In TGA, oxidative mass losses are the most often seen losses. It is crucial to research copper alloys' resistance to oxidation.

The National Aeronautics and Space Administration (NASA) is investigating improved copper alloys for potential application in combustion engines. However, because copper oxides form in oxygen-rich atmospheres, oxidative deterioration may occur in these alloys. Because NASA wants to be able to reuse shuttle materials, resistance to oxidation is important. For practical purposes, TGA can be used to investigate the static oxidation of materials like these. Distinct traces in the generated TGA thermograms indicate combustion during TG analysis. Samples of as-produced, unpurified carbon nanotubes with a high concentration of metal catalyst are one intriguing example. A TGA trace may diverge from the typical form of a well-behaved function due to combustion. A sudden change in temperature is the cause of this occurrence.

Plotting the weight and temperature over time reveals a sharp slope change in the first derivative plot that coincides with the sample's mass loss and the thermocouple's abrupt temperature rise. Beyond the oxidation of carbon brought on by poorly managed weight loss, the mass loss may be the consequence of smoke particles emitted during burning due to irregularities in the material itself. The anisotropy of the sample can also be diagnosed using different weight losses on the same sample at different times. For instance, sampling the top and bottom sides of a sample with dispersed particles can be useful in detecting sedimentation. This is due to the fact that if the particle distribution differs from side to side, thermograms will not overlap but rather display a gap between them.

Thermogravimetric kinetics

Thermogravimetric kinetics can be used to better understand the reaction mechanisms of thermal (catalytic or non-catalytic) breakdown involved in the pyrolysis and combustion processes of different materials. The activation energy of the breakdown process can be determined using the Kissinger technique. While a constant heating rate is more common, a constant mass loss rate can provide insight into some reaction dynamics. For instance, a constant mass loss rate of 0.2 weight percent per

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minute was used to determine the kinetic parameters of the carbonization of polyvinyl butyral.

12. X-ray diffraction [33]

Events pertaining to changes in the direction of X-ray beams caused by interactions with the electrons around atoms are collectively referred to as X-ray diffraction. It occurs due to elastic scattering when the energy of the waves remains unchanged. The resulting map of the X-ray directions that are distant from the material is called a diffraction pattern. It is not the same as X-ray crystallography, which determines the arrangement of atoms in materials using X-ray diffraction. It also has other components, like techniques for associating atomic positions with experimental diffraction observations.

Principle:

Crystals are regular groupings of atoms, while X-rays are electromagnetic waves. Atoms scatter X-rays, primarily through their electrons. Similar to how an ocean wave striking a lighthouse produces secondary circular waves from the lighthouse, an X-ray striking an electron produces secondary spherical waves from the electron. This phenomenon, known as elastic scattering, refers to the electron (or lighthouse) as the scatterer. A regular array of scatterers generates a regular array of spherical waves. Due to destructive interference, these waves cancel each other out in most directions but add constructively in a few specific directions.

An intuitive understanding of X-ray diffraction is provided by the Bragg model of diffraction. This method links a specific reflection to a set of regularly spaced sheets that pass through the crystal, usually via the centers of the atoms in the crystal lattice. X-rays scattered from adjacent planes will combine constructively (constructive interference) when the angle θ between the plane and the X-ray results in a path-length difference that is an integer multiple n of the X-ray wavelength λ. This pattern was proposed by William Lawrence Bragg, who assumed that incoming X-rays are scattered specularly (mirror-like) from each plane. The three Miller indices (h, k, and l) and their spacing (d) indicate the orientation of a specific set of sheets.

$$2d\sin\theta = n\lambda$$

A reflection is said to be indexed if its Miller indices (or, more precisely, its reciprocal lattice vector components) can be calculated using the known wavelength and the scattering angle 2θ. This indexing provides the dimensions, lengths, angles, and space group of the unit-cell.

13. Scanning electron microscope (SEM) [34-36]

By using a concentrated electron beam to scan a sample's surface, a scanning electron microscope (SEM) creates images of the material. The sample's atoms and electrons interact to produce a variety of signals that reveal details about the composition and topography of the surface. An image is created by scanning the electron beam in a raster scan pattern and combining the beam's position with the detected signal's intensity. A secondary electron detector (Everhart–Thornley detector) is used in the most popular SEM mode to detect secondary electrons released by atoms stimulated by the electron beam.

Among other factors, specimen topography affects the quantity of secondary electrons that can be found and, consequently, the signal intensity. Resolutions better than one nanometer are possible with some SEMs. Using specialized equipment, specimens are viewed at a variety of cryogenic or higher temperatures, in low vacuum or wet circumstances in a variable pressure or environmental SEM, or in high vacuum in a typical SEM.

Principle

Atoms at different depths within the sample interact with the electron beam to produce the signals that a SEM uses to create an image. Secondary electrons (SE), reflected or back-scattered electrons (BSE), distinctive X-rays and light (cathodoluminescence) (CL), absorbed current (specimen current), and transmitted electrons are among the signals that are generated. Although back-scattered electron detectors and secondary electron imaging are common methods in a SEM, other detectors could be employed to pick up more signals. For example, energy dispersive X-ray spectroscopy may detect emitted X-rays.

In solid matter, secondary electrons' mean free route is limited by their extremely low energies, which are around 50 eV. As a result, just the top few nanometers of a sample's surface allow SEs to escape. Images of the sample surface with a resolution of less than 1 nm can be obtained because the signal from secondary electrons tends to be extremely localized at the site of impact of the initial electron beam. Beam electrons that are reflected from the sample by elastic scattering are known as back-scattered electrons (BSE). The resolution of

BSE images is worse than that of SE images because they arise from deeper parts of the material due to their significantly higher energy.

However, because the intensity of the BSE signal is closely correlated with the atomic number (Z) of the material, BSE are frequently utilized in analytical SEM together with the spectra created from the characteristic X-rays. The distribution of the various components in the sample, but not their identities, can be inferred from BSE images. Colloidal gold immuno-labels with a diameter of 5 or 10 nm that would be hard or impossible to detect in secondary electron pictures can be imaged using BSE imaging in samples that are primarily made up of light elements, such biological specimens.

When an electron from the sample's inner shell is removed by the electron beam, a higher-energy electron fills the shell and releases energy, producing characteristic X-rays. Energy-dispersive X-ray spectroscopy or wavelength-dispersive X-ray spectroscopy may detect the energy or wavelength of these distinctive X-rays, which can be used to map the distribution of the elements in the sample and determine their abundance.

SEM micrographs feature a significant depth of field and a distinctive three-dimensional look that is helpful for comprehending a sample's surface structure because of the extremely narrow electron beam. The pollen micrograph above serves as an example of this. There are several different magnifications that can be achieved, ranging from roughly 10 times (roughly the same as a strong hand lens) to over 500,000 times, or over 250 times the maximum magnification of the best light microscopes.

14. Thin Layer Chromatography [37]

Principle:

TLC consists of two phases: a fixed phase and a mobile phase. Separation is the consequence of the analyte being divided between the stationary and mobile phases. Thin coating adsorbent substance, such as silica or cellulose, is glued to a glass plate or a thick piece of aluminium foil to form the stationary phase. The liquid eluent that makes up the mobile phase is composed differently depending on the type of separation needed. The eluent is often a combination made up primarily of organic substances, but it may also include water and other elements like acids to maintain the pH. Using a capillary or a micro-pipette, the analyte is spotted onto the TLC plate's base. The applied volume is often only a few µl. To make sure the analytes are concentrated in a tiny spot size, do this. The mobile phase reservoir is then inserted into the sealed chamber with the base of the TLC plate. The chamber is sealed to permit vapour phase saturation and stop solvent leakage

Applications of TLC

- **Sample purity:**

  Utilising TLC, one can determine a sample's purity. The sample and standard are directly compared; if any impurities are found, they will show up as extra spots and can be quickly identified.
- **Detection of compounds:**

  TLC can be used to purify, isolate, and identify natural materials such volatile oil or essential oil, fixed oil, waxes, alkaloids, glycosides, steroids, etc.
- **A look at the responses**

  The reaction mixture can be examined using TLC to see if the reaction is finished. This method is also employed to assess the effectiveness of various purification, molecular distillation, and distillation processes.
- **Biochemical testing**

  TLC is a powerful tool for removing unwanted substances from biological fluids including blood plasma, serum, urine, etc.
- **In order to separate and identify compounds that are structurally similar to one another:**

  In the discipline of chemistry, the TLC technique is being employed more frequently. It is used to identify cations and anions in inorganic chemistry.
- **The field of pharmaceutics**

  It is utilized by a number of pharmacopoeias for the identification of impurities in pharmacopoeial chemicals. The TLC technique has been used to conduct qualitative tests on a wide range of medications.
- **One of TLC's most crucial applications is the separation of multicomponent Medicinal formulations**

  Ingredients used in the food and cosmetics sectors, including as colours, preservatives, sugar replacements, and other sweeteners, are separated and identified using TLC.

Conclusion:

The atomic environment and carbon-hydrogen connection are determined using a nuclear magnetic resonance (NMR) spectrometer. The molecular weight, structure, and fragmentation patterns are identified using a mass spectrometer (MS). A Fourier Transform Infrared (FTIR) Spectrometer is used to determine whether a molecule has functional groups. Electronic

transitions and conjugation are analyzed using a UV-visible spectrophotometer. Heat-stable, volatile mixtures are separated using gas chromatography (GC). Complex non-volatile mixtures are separated and quantified using High-Performance Liquid Chromatography (HPLC). Compounds that have been isolated are identified using GC-MS and LC-MS. Crystalline structure is determined via X-ray diffraction (XRD). Optical activity is measured with a polarimeter. Stability and thermal characteristics are examined using thermal analyzers (DSC/TGA). For single particle examination, a scanning electron microscope (SEM) equipped with EDX is utilized. TLC is used for purity testing, compound identification, and reaction monitoring.

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We declare that there are no conflicts of interest. The writing and content of this article are solely our responsibility.

References:

[1]. C.K.Kokate, A.P.Purohit, S.B.Gokhale, Pharmacognosy, 57th edition, Alkaloids, Page number, 15.2 – 15.8, Chromatographic methods page number, 7.26-7.32 and Spectrophotometric methods Page number 7.32-7.37.

[2]. Y.R.Sharma, Elementary Organic Spectroscopy, Principles and Chemical Applications, Ultra violet and visible spectroscopy, page no 16-18, Nuclear Magnetic Resonance spectroscopy, page no 198, 13C NMR spectroscopy, page no 242-243, Mass spectrometry, page number 292-295.

[3]. De Hoffmann, Edmond, Stroobant, Vincent, Mass spectrometry (Principles and Applications) second edition, Wiley, page no 157-158,2002.

[4]. Pitt James J, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry”, Clin Biochem Rev, 30(1), 19-34, 2009.

[5]. Ressom Habtom W, Tuli Leepika, Xiao Jun Feng, Zhou Bin, “LC-MS-based metabolomics” Mol.Biocyst, 8(2), 470-481, Feb, 2012.

[6]. Chaimbault Patrick, “ The modern Art of Identification of Natural Substances in Whole Plants” Recent Advances in Redox Active Plant and Microbial Products, Springer Netherlands, page number 31-94, 01-01-2014.

[7]. Dass chhabil, “Hypenated Separation Techniques”, Fundamentals of Contemporary Mass Spectrometry, John Wiley & Sons, Inc, page no 151-194, 01-01-2007.

[8]. Salzmann Linda, Seger Christoph, “ After another decade, LC-MS/MS became routine in clinical diagnostics”, Clinical Biochemistry, Advancement and Applications of Mass spectrometry in Laboratory Medicine, 82,2-11, 01-08-2020.

[9]. Pitt James J, “Principles and Applications of Liquid Chromatography-Mass Spectrometry in Clinical Biochemistry”, the Clinical Biochemist Reviews, 30(1), 19-34, 12-03-2017.

[10]. Niessen Wilfried M.A, Liquid chromatography-Mass Spectrometry, Third edition, Boca Raton, CRC Taylor & Francis, page number 50-90, 2006.

[11]. Lenahan R, Wang, “Determination of volatile halocarbons in water by purge-closed das chromatography”, Bulletin of Environmental contamination and Toxicology. 32(4), 429-438, April, 1984.

[12]. Ellis RD, Mc Lafferty, Peterson DW, Stauffer DB, Probability Based Matching of Mass Spectra, Rapid Identification of Specific Compounds in Mixtures, Organic Mass Spectrometry, 9(4), 690-703, 1974.

[13]. Amirav A, Fialkov AB, Gordin A, Poliac M, “Gas chromatography-Mass spectrometry with supersonic molecular beams” Journal of Mass Spectrometry, 43(2), 141-163.

[14]. Alon T, Amirav A, Isotope abundance analysis method and software for improved sample identification with supersonic gas chromatography/mass spectrometry”, Rapid Communications in Mass Spectrometry, 20(17), 2579-2588.

[15]. Hubschmann HJ, Handbook of GC-MS, Fundamentals and Applications, (3ed), John Wiley & Sons, Incorporated, P.731, 22 April 2015.

[16]. Aggelis Y, Fragkaki, Kioukia-Fougia N, Kiousi X, Lyris E, Tsivou M, An overview of the doping control analysis during the Olympic Games of 2004 in Athens, Greece", Analytica Chemica Acta 555, 1-13, 2006.
[17]. Hart, C., Organische Chemie, Wiley-VCH, 2002,ISBN 3-527-30379-0
[18]. *Carey, F. A.; R. J. Sundberg, Advanced Organic Chemistry, Part A: Structure and Mechanisms (Fifth ed.). Springer.2007, p. 123*
[19]. Yaragalla, Srinivasarao; Mishra, Raghvendra Kumar; Thomas, Sabu; Kalarikkal, Nandakumar; Maria, Hanna J, Carbon-Based Nanofillers and Their Rubber Nanocomposites. Elsevier Science,11 February 2019.
[20]. Missal W, Kita J, Wappler E, Gora F, Kipka A, Bartnitzek T, Bechtold F, Schabbel D, Pawlowski B, Moos R, "Miniaturized Ceramic Differential Scanning Calorimeter with Integrated Oven and Crucible in LTCC Technology". Procedia Engineering, Elsevier: 2010, 5, 940–943.
[21]. Hohne G, Hemminger WF, Flammersheim HJ, Differential Scanning Calorimetry, Springer-Verlag, 2023,pp. 17 ff.
[22]. Liu, X.; Yu, W. "Evaluating the Thermal Stability of High Performance Fibers by TGA", Journal of Applied Polymer Science, 2006, 99 (3): 937–944.
[23]. Lopresti, Mattia; Alberto, Gabriele; Cantamessa, Simone; Cantino, Giorgio; Conterosito, Eleonora; Palin, Luca; Milanesio, Marco. "Light Weight, Easy Formable and Non-Toxic Polymer-Based Composites for Hard X-ray Shielding: A Theoretical and Experimental Study". International Journal of Molecular Sciences, January 28, 2020, 21 (3): 833.
[24]. Lopresti, Mattia; Palin, Luca; Alberto, Gabriele; Cantamessa, Simone; Milanesio, Marco, "Epoxy resins composites for X-ray shielding materials additivated by coated barium sulfate with improved dispersibility". Materials Today Communications, 20 November 2020, **26,** 101888.
[25]. Reyes-Labarta J.A, Marcilla A, "Kinetic Study of the Decompositions Involved in the Thermal Degradation of Commercial Azodicarbonamide" (PDF). Journal of Applied Polymer Science, 2008, 107 (1): 339–346.
[26]. Marcilla, A, Gómez, A, Reyes, J.A, "MCM-41 Catalytic Pyrolysis of Ethylene-Vinyl Acetate Copolymers. Kinetic Model". Polymer, 2001, 42 (19): 8103–8111.
[27]. Marcilla, A, Gómez, A, Reyes-Labarta, J.A.; Giner, A. (2003). "Catalytic pyrolysis of polypropylene using MCM-41. Kinetic model". Polymer Degradation and Stability,2003, 80 (2): 233–240. .
[28]. Marcilla A, Gómez, A, Reyes-Labarta, J.A, Giner A, Hernández, F, "Kinetic study of polypropylene pyrolysis using ZSM-5 and an equilibrium fluid catalytic cracking catalyst",Journal of Analytical and Applied Pyrolysis, 2003, 68–63: 467–480.
[29]. Conesa J.A, Caballero J.A, Reyes-Labarta J.A, "Artificial Neural Network for Modelling Thermal Decompositions". Journal of Analytical and Applied Pyrolysis, 2004, 71 (1): 343–352. .
[30]. Reyes, J.A.; Conesa, J.A.; Marcilla, A. (2001). "Pyrolysis and combustion of polycoated cartons recycling. kinetic model and ms analysis". Journal of Analytical and Applied Pyrolysis, 2001, 58–59: 747–763.
[31]. Janeta, Mateusz, Szafert, Sławomir, "Synthesis, characterization and thermal properties of T8 type amido-POSS with p-halophenyl end-group". Journal of Organometallic Chemistry. Organometallic Chemistry: from Stereochemistry to Catalysis to Nanochemistry, 2017, 847 (Supplement C): 173–183.
[32]. Tikhonov N. A, Arkhangelsky I. V, Belyaev S. S, Matveev A. T, "Carbonization of polymeric nonwoven materials", Thermochimica Acta, 2009, 486 (1–2): 66–70.
[33]. Cullity B. D, Elements of x-ray diffraction. Stuart R. Stock (3rd ed.), Upper Saddle River, NJ, Prentice Hall, 2001.
[34]. Stokes, Debbie J, Principles and Practice of Variable Pressure Environmental Scanning Electron Microscopy (VP-ESEM), Chichester, John Wiley & Sons, 2008.
[35]. McMullan D, "Scanning electron microscopy 1928–1965". Scanning, 2006, 17 (3): 175–185.
[36]. Suzuki E, "High-resolution scanning electron microscopy of immunogold-labelled cells by the use of thin plasma coating of osmium". Journal of Microscopy, 2002, 208 (3): 153–157.

[37]. Rameshwar S Cheke, Rohan R.Narkhede, Sachin D.Shinde, Vaibhav S.Adhao, Instrumental methods of Analysis, Thin Layer Chromatography, page no 189-207.

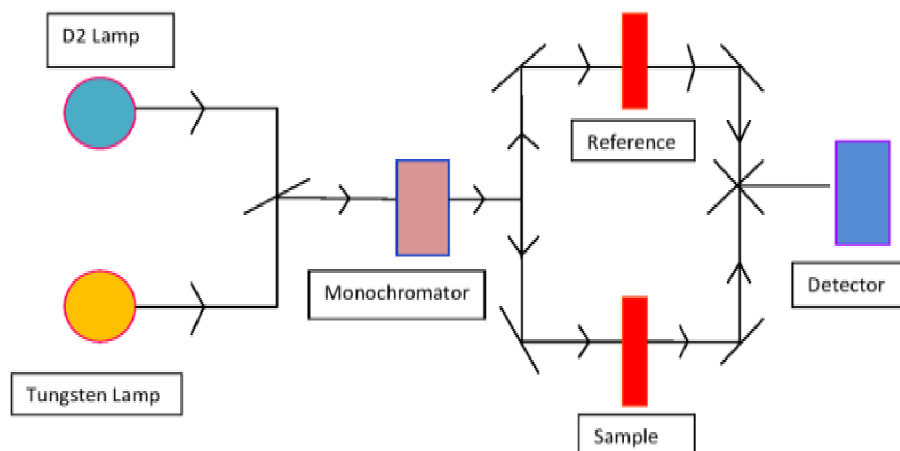

Fig 1: Ultraviolet-Visible spectrophotometer (Double beam)

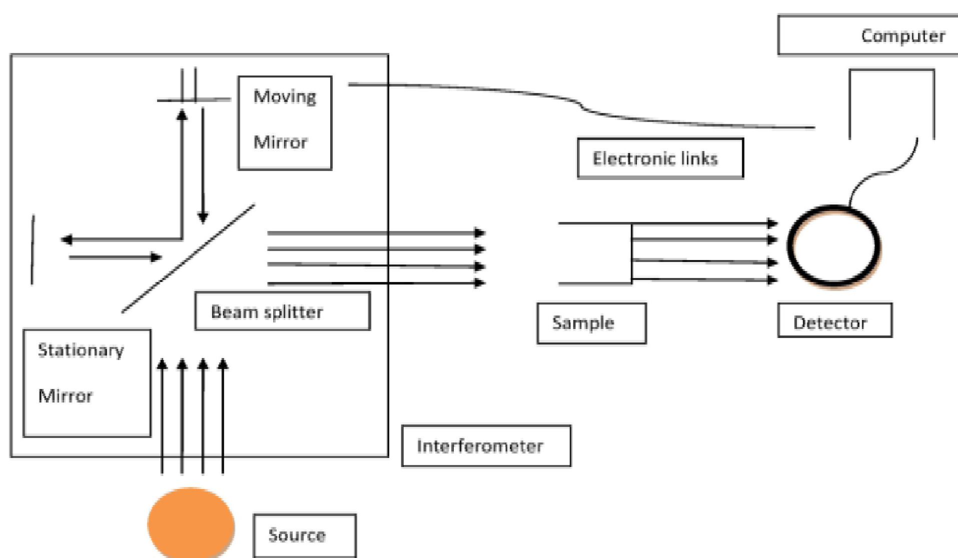

Fig2: Instrumentation schematic for FTIR

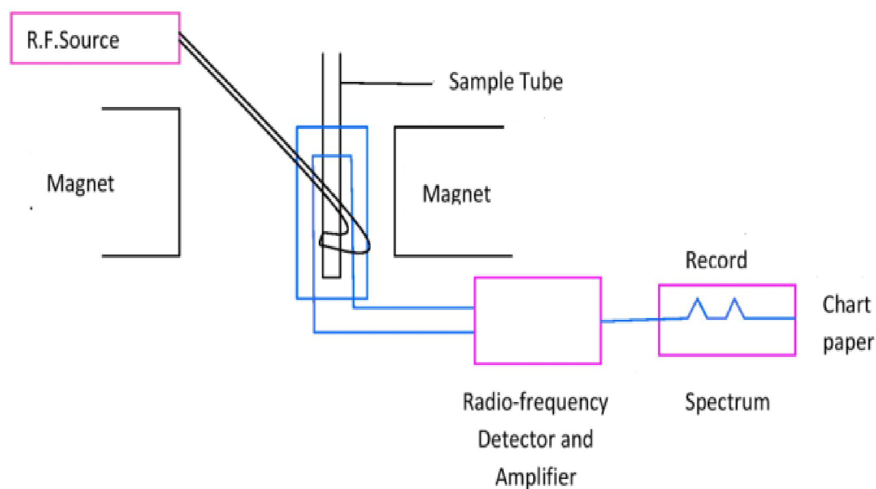

Fig3: Nuclear Magnetic Resonance Spectrometer

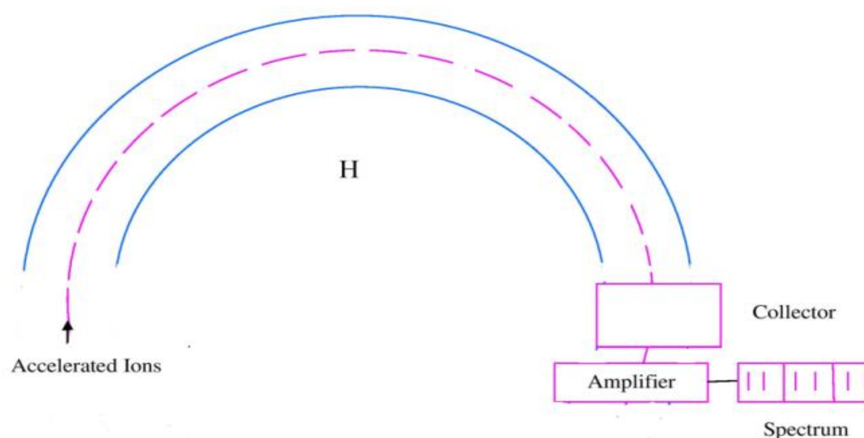

Fig 4: Mass spectrometer