UNIT 13 INSTRUMENTATION IN FOOD ANALYSIS

Structure

13.0 OBJECTIVES

After reading this unit, we shall be able to:

- reason advanced instrumentation required in food analysis;
- outline principles of instrumental techniques used in food analysis; and
- describe applications of instrumental technique in analysis of macro and micro food components.

13.1 INTRODUCTION

13.1.1 Need for Food Analysis

Food analysis is the discipline dealing with the development, application and study of analytical procedures for characterizing the properties of foods and their constituents. All food products whether raw or processed are analyzed to provide information about a wide variety of different characteristics, including their composition, structure, physicochemical properties and sensory attributes. In fact the food is analyzed for a variety of reasons, e.g. compliance with legal and labeling requirements, assessment of product quality, determination of nutritive value, detection of adulteration, research and development.

Food safety is an issue of prime importance. With the growing concerns about the food and health safety, the food regulatory authorities in different countries have imposed stringent mandatory norms for the presence of various toxicants, which if present beyond a prescribed residual level might prove hazardous to human health. Moreover, with the implementation of WTO and globalization, it has become important that all food products for export out of the country should meet the regulatory norms of the prescribed limits of different toxicants in various food products. As, government bodies regulate the permitted levels of contaminant compounds; much of this advancement has been driven by increased sensitivity and specificity of determination technique e.g. using analytical instruments.

Food Analysis serves as a unique and invaluable tool for all food scientists, technologists and regulatory authorities for quality assurance and control of food products, to study the different aspects of food products.

Food is a complex matrix consisting of different components. These components can be categorized into different categories which are listed as given below:

1) **Nutrients:** e.g. Proteins, Amino acids, Total cholesterol, Trans Fats and Lipid profile, Carbohydrates, Sugars, Dietary fiber, Vitamins, Minerals etc. Depending upon the food product some of them may be present at high concentration levels while others may be present at low concentration levels of parts per million.

2) **Additives:** e.g. Colors, Dyes, Stabilizers, Antioxidants, Flavors and Fragrance, Preservatives, etc.

   The additives are added to the food products for the purpose of giving the food products desired appearance, texture, flavor and extending the shelf life. The additives are usually present at very low concentration levels.

3) **Adulterants:** They are added intentionally to the food products mostly for the purpose of cost benefits and they may be present at higher as well as lower amounts. They may be safe or sometimes highly toxic, such as, argemone in mustard oil, sudan red in chillies, animal cholesterol in ghee, low cost vegetable oil in high cost vegetable oil etc.

4) **Contaminants and Toxicants**: Toxicants can be classified into Physical toxicants (e.g. glass, wood, metal, insect matter etc.); Biological toxicants such as microbes and pathogens; and Chemical toxicants such as residual pesticides, residual antibiotics, mycotoxins, and environmental pollutants like PAH (polycyclic aromatic hydrocarbons), PCB (polychlorinated

biphenyls), Dioxins, toxic metals etc. Most of the times these contaminants are not added intentionally but find there way into the food products from environmental pollution or if proper practices are not being followed during agriculture, animal breeding, storage or processing. The various toxicants are present at low levels of concentration and if present beyond a certain prescribed level of concentration in food products may prove to be highly toxic or carcinogenic to humans.

13.1.2 Why do We Need Instrumentation in Food Analysis?

Due to complex nature of food matrix, it often becomes impossible to accurately analyze one component in the presence of others using the classical method of analysis. More often than not, interferences are encountered during the measurement of minor components in the presence of the components present in bulk quantities. All this may lead to inaccurate and unreliable results and sometimes erroneous and false results because of lack of specificity and sensitivity of classical method. Therefore, in order to achieve the reliability of results, today the instrumental analytical techniques have become mandatory in development, quality control and safety, exports of food products and meeting the regulatory norms of food products.

13.2 SELECTING AN APPROPRIATE INSTRUMENTAL TECHNIQUE

There are usually a number of different analytical techniques available to determine a particular property of a food material. It is therefore necessary to select the most appropriate technique for the specific application. The analytical technique selected depends on the property to be measured, the type of food to be analyzed and the reason for carrying out the analysis.

13.2.1 Criteria for Selecting a Technique

Some of the criteria that are important in selecting an instrumental analytical technique are listed below:

- **Precision:** A measure of the ability to reproduce a result by a specific analyst (or group of analysts) using the same equipment and experimental approach kepping other conditions unchanged.
- **Reproducibility:** A measure of the ability to reproduce result using the same experimental approach in same as well as different laboratories using same/different equipment.
- **Accuracy:** A measure of how close one can actually measure the value to the true value of the parameter being measured.
- **Simplicity of operation:** A measure of the ease with which relatively unskilled workers may carry out the analysis.
- **Speed:** Analysis of single sample or the number of samples in a given time.
- **Sensitivity:** A measure of the lowest concentration of the component that can be detected by a given procedure.
- **Specificity:** A measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components e.g., Fructose in the presence of sucrose or glucose.

- **Nature of food matrix:** The composition, structure and physical properties of the matrix material surrounding the analyte often influences the type of method that can be used to carry out an analysis e.g. whether the matrix is solid or liquid, transparent or opaque, polar or nonpolar.

If there are a number of alternatives methods available for measuring a certain property of a food, the choice of a particular method will depend on which of the above criteria is most important.

13.2.2 Instrumental Techniques in Food Analysis

Analysis of food products for the majority of the parameters can be undertaken using different instrumental techniques as described below.

- **Chromatographic Techniques**
- **Hyphenated Techniques**
- **Spectroscopic Techniques**
- **Thermal methods of analysis**

13.3 CHROMATOGRAPHIC TECHNIQUES

Chromatography is defined as a process by which solutes are separated by a dynamic differential migration in a system consisting of two or more phases, one of which moves continuously in a given direction called as mobile phase and in which the individual substances exhibit different mobilities by reason of differences in adsorption, partition, solubility, vapour pressure, molecular size or ionic charge density. The individual substances thus separated can be identified or determined using appropriate technique. Chromatographic techniques can broadly be classified into:

1) Gas Chromatography (GC)

Gas chromatography is applied to volatile organic compounds. The mobile phase is a gas and the stationary phase is usually a liquid on a solid support or sometimes a solid adsorbent.

2) Liquid Chromatography (LC)

Liquid chromatography is used to separate analytes in solution including metal ions and organic compounds. The mobile phase is a solvent and the stationary phase is a liquid on a solid support, a solid, or an ion-exchange resin.

13.3.1 Gas Chromatography

Gas Chromatography (GC) is one of the most versatile analytical techniques used in the food industry. GC is used to separate volatile organic components in a mixture. It enables fast separation and identification of components in a complex mixture using appropriate detectors. Substances to be analysed by GC must be volatile and must be thermally stable below 350°C.

A schematic diagram of a gas chromatograph showing various components is presented in Fig. 13.1. Samples are rapidly injected by means of a hypodermic micro syringe through a silicone rubber septum into the column. The sample injection port, column, and detector are heated to temperatures at which the

sample has a vapour pressure of at least 10 torr, usually about 50ºC above the boiling point of the highest boiling solute. The injection port and detector are usually kept at higher temperature than the column to promote rapid vaporization of the injected sample and prevent sample condensation in the detector. For packed columns, liquid samples of 0.1 to 10 μL are injected. For capillary columns, volumes of only about 1/100 these sizes must be injected because of the lower capacity (albeit greater resolution) of the columns. Sample splitters are included on chromatographs designed for use with capillary columns that deliver a small fixed fraction of the sample to the column, with the remaining part going to waste.

The response is detected in the form of peaks. Separation occurs as the vapor constituents equilibrate and partition between carrier gas and the stationary phase. The carrier gas is a chemically inert gas available in pure form such as argon, helium, or nitrogen.

The sample is automatically detected as it emerges from the column (at/or a constant flow/ gas pressure rate), using a variety of detectors whose response is dependent upon the composition of the vapor. The chromatographic peaks are recorded as a function of time. By measuring the **retention time** (elapsed time in minutes between the time a sample is injected and the time the chromatographic peak reaches maximum intensity) and comparing this time with that of a standard of the pure substance, it may be possible to identify the peak (agreement of retention times of two compounds does not guarantee the compounds are identical). The area under the peak is proportional to the concentration, and so the amount of the substance can be quantitatively determined. The peaks are often very sharp and, if so, the peak heights can be compared with a calibration curve prepared in the same manner.

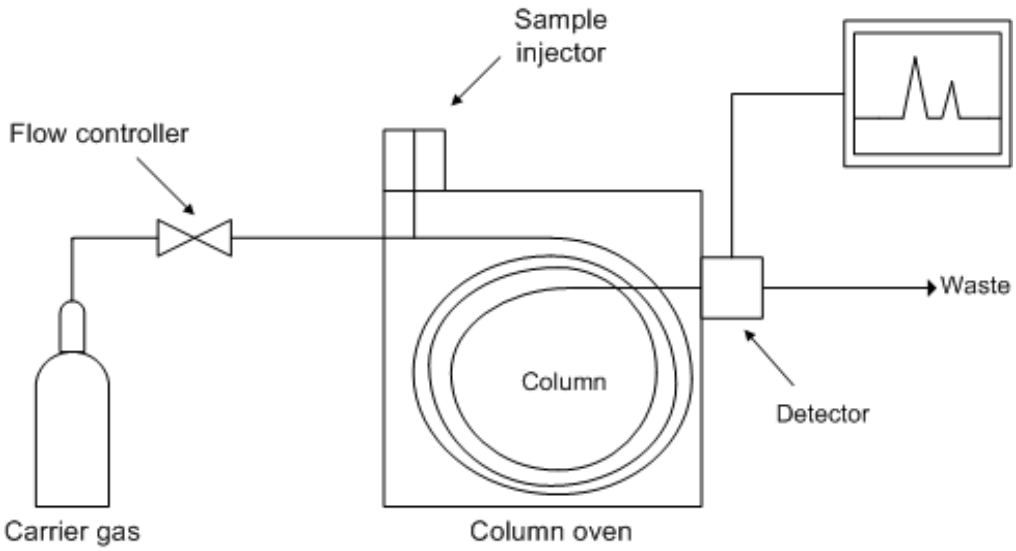

Fig 13.1: Schematic Diagram of a Gas Chromatograph

13.3.2 Detector for Gas Chromatography

Various types of detectors are used for different applications:

- Flame Ionisation Detector (FID)
- Electron Capture Detector (ECD)

- Nitrogen Phosphorous Detector (NPD)
- Thermal Conductivity Detector (TCD)

A) Flame Ionisation Detector (FID): FID is the most widely used of all detectors because, it is reliable, easy to maintain and operate, rugged and responds to almost all classes of compounds but, insensitive to water. The response is roughly dependent upon number of carbon atoms in the molecule. Hydrogen and air are the required gases for the ignition of flame.

An organic compound is burnt in a flame produced by H_2 and air which in turn produces ions.

Organic sample + H_2 + O_2 -----> CO_2 + H_2O + $(ions)^+$ + e^-

The ions produced are collected by a pair of polarized electrodes inside the detector and the current is produced which is directly proportional to the concentration. The current is amplified and then recorded.

B) Electron Capture Detector (ECD): ECD is the most sensitive detector for halogenated compounds, anhydrides, peroxide, conjugated carbonyls, nitrites, nitrates and organometallics, but, insensitive to hydrocarbons, alcohols, ketones and amines. Solvent containing analyte from the column is passed over a β (electron)-emitter (radioactive compound that emits electrons, e.g. ^{63}Ni). Electron from the emitter causes ionization of carrier gas producing a burst of electrons, which produce a constant current between a pair of electrodes. But, when a solute with higher electron effinity is eluted from the column, some of the electrons are captured and the current flow is reduced in proportion to the concentration of the eluting compound.

C) Nitrogen Phosphorous Detector (NPD): NPD is a selective detector for nitrogen and phosphorous containing organic compounds, e.g. organophosphorus and carbamate pesticides. NPD is similar in design to FID. In this detector nitrogen and phosphorus containing molecules exiting the column collide with electrically heated ceramic thermoionic bead which is positioned near the jet orifice and undergo catalytic surface chemical reaction. Ions created in this reaction are attracted towards a collector electrode, amplified and output is given to data system.

D) Thermal Conductivity Detector (TCD): This detector is used for the analysis of permanent gases. It responds to all gases and vapours with a thermal conductivity different from that of carrier. In this detector, no separate fuel is required. This detector consists of two pair of heated elements, each as an arm of a Wheatstone bridge over which the column effluent and a reference gas stream flow. When there is only reference gas, balance of conductivity in the bridge is maintained. But when the analyte reaches the filament, the thermal conductivity is changed, causing unbalanced bridge which provide signal.

13.3.3 Sampling Techniques

There are different types of sampling techniques normally employed for GC analysis of volatile samples:

1) Headspace analysis.

2) Thermal Desorption.

3) Purge and Trap technique

1) Headspace Analysis

A sample in a sealed vial is equilibrated at a fixed temperature, for example 10 min. and the vapour in equilibrium above the sample is sampled and injected into the gas chromatograph flavour analysis etc.

2) Thermal Desorption

Thermal Desorption (TD) is a technique in which solid or semisolid samples are heated under a flow of inert gas. Volatile and semi volatile organic compounds are extracted from the sample matrix into the gas stream and introduced into a gas chromatograph. Samples are typically weighed into a replaceable PTFE tube liner, which is inserted into a stainless steel tube for heating.

Thermal desorption is well suited for dry or homogenous samples such as food packaging films, spices, coffee flavour profile, voletile organics in wine, mushrooms, fruits, honey etc. solid foods, cosmetics, ointments, and creams.

3) Purge and Trap Technique

The Purge and Trap technique is a variation of thermal desorption analysis in which volatiles are purged from a liquid sample placed in a vessel by bubbling a gas (e.g. air or nitrogen) through the sample and collecting the volatiles in a sorbent tube containing a suitable sorbent. The trapped volatiles are then analyzed by thermally desorbing them from the sorbent. This is a form of 'Headspace' analysis in which analytes are concentrated prior to introduction into the GC.

Purge and Trap is suitable for non-homogenous samples, since fairly large and high moisture samples can be taken. Examples include foods such as pizza or fruits. The measurements of malodorous organic volatiles in the headspace vapor above a sample of stored food, is used to determine whether it still meets the "freshness" requirements.

13.3.4 Applications of Gas Chromatography

Gas Chromatography can be applied for analysis and determination of different compounds in food products such as :

1) Cholesterol, Fatty acid profiling and Trans fat analysis;
2) Antioxidants and Preservatives like TBHQ, Benzoic acid, Sorbic acid Acetic acid, etc;
3) Analysis of residual pesticides and environmental contaminants;
4) Characterization of flavours and fragrances; and
5) The Gas Chromatographic profiling of the essential volatile oils gives a reasonable 'fingerprint' which can be used to characterize the identity of the particular oil.

13.4 LIQUID CHROMATOGRAPHY

Liquid chromatography covers a variety of separation techniques such as liquid solid (adsorption chromatography), liquid-liquid (partition chromatography),

ion exchange, size exclusion, thin layer, high performance thin layer and paper chromatography; all involving a liquid mobile phase. In this unit, we would learn more about the liquid chromatographic techniques used specially for the purpose of food analysis i.e.

- High Performance Liquid Chromatography (HPLC)
- Thin Layer Chromatography (TLC)
- High Performance Thin Layer Chromatography (HPTLC)

HPLC is used to separate, identify, and quantify polar and non-volatile compounds when in mixture. HPLC utilizes a column that holds liquid stationary phase, a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules.

- **Normal phase chromatography**

In this technique, the stationary phase is polar. A non-polar mobile phase, such as n-Hexane, methylene chloride, or chloroform is used. The stationary phase is bonded silioxane with a polar functional group, e.g. Cyano, diol, amino, dimethylamino, etc. These phases retain polar compounds in preference to non-polar compounds.

- **Reversed phase chromatography**

In Reversed Phase Chromatography (RPC) relatively non-polar stationary phase is used, with a polar mobile phase constituting of methanol, acetonitrile, tetrahydrofuran, etc., usually in combination with water. Methanol and acetonitrile are most common. The water content is varied for adjusting the polarity. Methanol is used for acidic compounds and acetonitrile for basic compounds. Tetrahydrofuran is used for those with larger dipoles. These solvents are UV transparent and have low viscosity. The most common bonded phases are n-octaldecyl (C_{18}) or n-octyl (C_8) bonded polysiloxanes.

13.4.1 Characteristic Features of HPLC

- Fast, accurate and high power of resolution.
- Results are repeatable and reproducible.
- Facilitates determination of multiple components in a single run.
- Provides method of choice for thermally unstable and high molecular weight compounds.
- Separated components can be easily collected from the mobile phase for further characterization, as it is a non-destructive technique.
- Suitable for both aqueous and non-aqueous sample.

13.4.2 Comparison of HPLC and GC

HPLC in some respect is more versatile then GC since:

1) It is not limited to volatile and thermally stable substances. It can accommodate thermally unstable, nonvolatile compounds and inorganic ions.
2) The choice of mobile and stationary phase is wider.

GC is better from the point of view of speed and simplicity of equipment. The analysis cost is also significantly cheaper as HPLC requires highly pure solvents that are costly.

HPLC is an excellent technique for separation of chemical compounds with high degree of specificity and selectivity and is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, as in the case classical column chromatography it is forced through under high pressures of up to 400 atm, which makes it much faster. The technique is not limited by the volatility or stability of the sample compound.

13.4.3 A Typical Modern Liquid Chromatograph

A Typical Modern Liquid Chromatograph Consists of:

i) Solvent delivery system which includes a pump

ii) Sample injection system

iii) Column and Detector

iv) Chromatography software and Computer

Compounds are separated by injecting a sample mixture into the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile and the stationary phase. As the sample solution flows through the column with the mobile phase, the components present in the injected sample solution migrate according to the non-covalent interactions of the compounds with the column. The mobile phase could be run in isocratic and gradient modes. A separation by HPLC, which employs constant composition of mobile phase throughout the chromatographic run, is called an isocratic elution and in case two or more solvents of different polarities are used in different proportions during the chromatographic run the method is known as gradient elution.

The **stationary phase** in HPLC refers to the solid support contained within the column through which the mobile phase continuously flows. Columns containing various types of stationary phases are available commercially. For most of the separation in food analysis the columns normally used are Normal phase (e.g. silica) or the Reverse phase (e.g. C_8, C_{18}). Samples are injected into the HPLC via an injection port. In modern HPLC systems, the sample injection is typically automated.

13.4.4 Detectors for HPLC

In order to detect the compounds as they elute from the column there are many types of detectors that can be used with HPLC. Some of the common detectors include: Ultra Violet (UV), Photo Diode Array (PDA), Refractive Index (RI), Fluorescent, and Mass Spectrometric (MS) detector.

A) Refractive Index (RI): Detectors measure the ability of sample molecules to bend or refract light. This property for each molecule or compound is called its refractive index. For most RI detectors, light proceeds through a bi-modular flow-cell to a photo detector. One channel of the flow-cell directs the mobile phase passing through the column while the other directs only the mobile phase. Detection occurs when the light is refracted due to samples eluting from the column, and this is read as a disparity between the two channels.

B) Ultra-Violet (UV): Detector measure the ability of a sample to absorb radiation in the UV region. This can be accomplished at one or several wavelengths.

C) Photo Diode Array (PDA): Detector produces a three-dimensional graph that assists in examining the purity of a peak. The chromatographic peak is supported by authentication through UV-VIS spectrum.

D) Fluorescent: Detector measure the ability of a compound to absorb and then re-emit light at given wavelengths. Each compound has a characteristic fluorescence. The excitation source passes through the flow-cell to a photodetector while a monochromator measures the emission wavelengths.

E) Mass Spectroscopy (MS) Detectors: The sample compound or molecule is ionized, passed through a mass analyzer, and the ion current is detected. There are various methods for ionization.

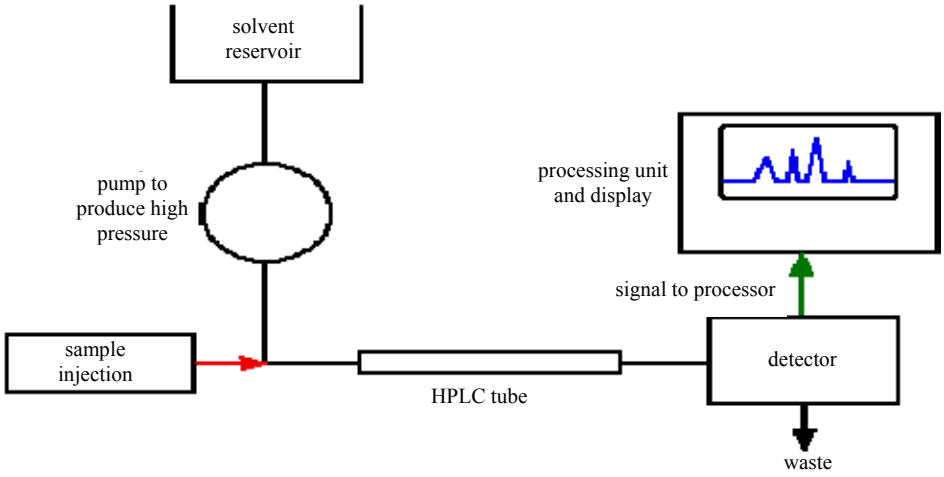

Fig. 13.2: Schematic Diagram of HPLC

13.4.5 Applications of HPLC

HPLC finds wide applications in food, both for profiling and analysis of various components such as:

1) Amino acids profiling, peptides and hproteins.

2) Lipids and alcohols.

3) Carbohydrates and carbohydrate profiling, sweeteners.

4) Fat soluble and water soluble vitamins, carotenoids.

5) Organic acids and organic bases.

6) Residues of Mycotoxin, Antimicrobial and veterinary drugs, pesticides, etc.

7) Pigments, colorants and phenolic compounds.

8) Bittering substances.

9) Additives, preservatives, antioxidants and stabilizers in processed food products.

13.5 THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is an important chromatographic technique, frequently used for qualitative identification and initial screening of components. A sample is spotted onto the plate or a strip with a micropipette and the plate or the strip is placed in a suitable solvent to develop the chromatogram. The solvent is drawn up the plate by capillary action, which moves the sample components up the plate at different rates, depending upon their solubility and their degree of retention by the stationary phase. Following development, the individual spots are noted or made visible by treatment with a reagent that forms a coloured derivative. For example, amino acids and amines are detected by spraying the plate with a solution of ninhydrin, resulting in a blue or purple coloured spot. If the solute compound is fluorescent, they can be detected by exposing to UV light. The spots generally move at a certain fraction of the rate at which the solvent moves and they are characterized by the R_f value.

$$R_f = \frac{\text{Distance solute moves from the point of application}}{\text{Distance solvent front moves}}$$

R_f value is characteristic for a given stationary phase and solvent combination. Since the separation and identification spots on TLC is based upon visual observation, at certain times, if the product analyzed contains large number of components, the method may suffer from poor resolution due to the closely lying or the overlapping spots and poor specificity. Therefore, the results may sometimes be uncertain, misleading or inaccurate.

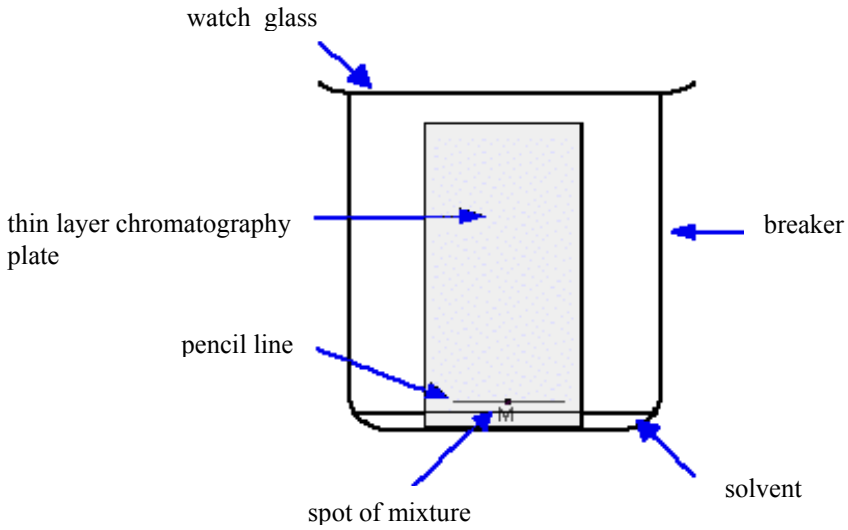

Fig. 13.3: Schematic Diagram of a thin Layer Chromatographic Technique

13.6 HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC enhances the power of thin layer chromatography by improving the speed and efficiency of separation by the development of instrumentation to automate sample application, development of the chromatogram, and detection, including accurate and precise *in situ* quantitation.

HPTLC is a selective, precise, and accurate chromatographic technique for fingerprint analysis of food products. This involves densitometric evaluation after resolving the components of the sample on silica gel plates with very fine

particle size. For densitometric evaluation, peak areas are recorded at the appropriate wavelength. HPTLC has the advantages of many fold possibilities of qualitative and quantitative detection in analyzing food products with accuracy and precision.

Applications

HPTLC can be used for a large number of applications in food industry for screening purposes, e.g.:

- Determination adulterants in food products such as argemone in mustard oil.
- Separation of food colours in food samples.
- Determination of residues of mycotoxins in food products.

Check Your Progress Exercise 1

Note: a) Use the space below for your answers.
b) Check your answers with those given at the end of the unit.

1) Define Chromatography technique?

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2) Explain the applications of Gas Chromatography?

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3) Name the detectors used for HPLC and GC.

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13.7 HYPHENATED TECHNIQUES

In the past two decades, combining chromatographic separation system on-line with a spectroscopic detector in order to obtain structural information on the analytes present in a sample has become the most important approach for the identification and/for confirmation of the identity of target and unknown chemical compounds. For most (trace-level) analytical problems in research field of food products, the combination of column liquid chromatography or

capillary gas chromatography with a mass spectrophotometer has become the preferred approach for the analysis of food products. Two very important examples of hyphenated procedures that are used in food analysis are GC-MS and LC-MS. The information obtained from GC-MS or LC-MS is not only useful for quantitation but also provide confirmation of the analyte, specially if the residue is present at trace level. In GC and HPLC analysis, the results sometimes might be misleading as compounds having similar chemical nature often elute at approximately the same retention time.

Mass spectrometry is an analytical technique that identifies the chemical composition of a sample on the basis of the mass to charge ratio of charged particles. The technique has both qualitative and quantitative uses, including:

- Identifying unknown compounds by the mass and mass fragmentation pattern, which are usually specific in nature. Identification is also possible on comparison to standard mass spectrometry libraries, which usually accompanies GC-MS instruments. In addition, it is also possible to develop own library of standard reference compounds that can be utilized in identifying compounds in unknown samples.
- Determining the isotopic composition of elements in a compound.
- Determining the structure of a compound by observing its fragmentation pattern.
- Quantifying the amount of a compound.

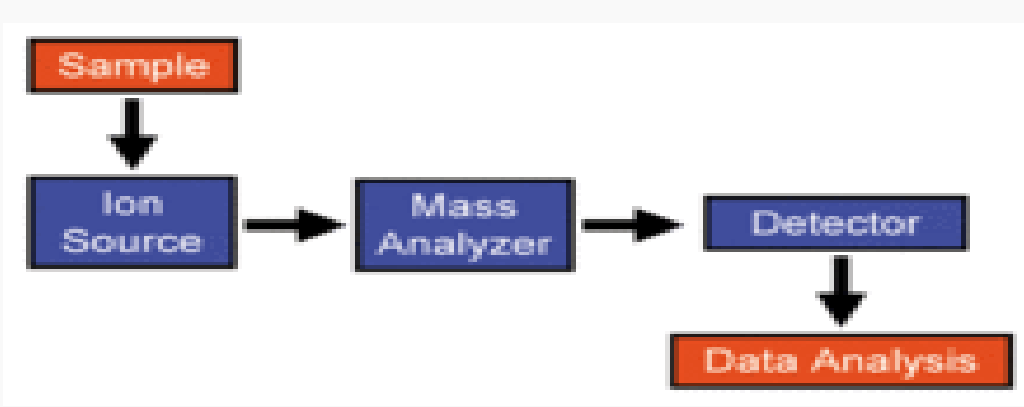

Fig. 13.4: Schematic Diagram of GC-MS

The underlying principle of mass spectrometry is that as charged particles ions pass through electric and magnetic fields, their paths vary according to their mass-to-charge ratios. Devices which operate according to this principle are called mass spectrometers. The design of a mass spectrometer has three essential modules: an **ion source**, which transforms the molecules of a sample into a variety of ionized fragments; an **analyzer**, which sorts the ions by their masses, using internally generated electric and magnetic fields; and a **detector**, which measures the value of some indicator quantity and thus providing data for calculating the abundances of respective ion fragments.

13.7.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The capabilities of integrated gas chromatography-mass spectrometry are almost unique in meeting the requirements for analytical methods which are

not only highly sensitive but also specific and reliable in providing information on specific compounds as a function of their concentrations.

The GC portion of this system provides separation of volatile organic solutes in a mixture in the gas phase. As each solute exits the GC column, it is diverted into a mass spectrometer which is capable of monitoring both the amount and identifying the chemical nature of the solute. In this way, both quantitative and qualitative information about the mixture can be obtained.

The MS portion of the system takes each gaseous solute eluting out of gas chromatograph and ionizes it in an electron beam. The ions formed by a specific solute will depend on the nature of the bonds in the molecule, and it is possible to get both ionized molecules and its fragments. The ions thus formed are then directed down a separator which isolates and counts the ions according to mass. The sequence and relative intensity of the mass peaks give information about the chemical identity of the solute. The absolute intensity of the peaks provide information about the amount of substance present.

Applications of Gas Chromatography Mass spectrometry:

Gas chromatography is useful for a number of applications in food analysis specially from the point of view of safety of the food products. The technique is useful for:

1) Pesticide residue analysis in all raw and processed food products.
2) Analysis of environmental contaminants, such as polyclorinated biphenyls, polyaromatic hydrocarbons, dioxins, etc. in food products.
3) Fatty acid profiling in oils and fats.
4) Flavours and fragrance in food products.
5) Volatiles and other residual solvents in food packaging material.

13.7.2 Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry (**LC-MS**) is an analytical technique that combines the physical separation capabilities of HPLC coupled with the confirmation by mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally, its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals in a complex mixture.

LC-MS technique is used when very high sensitivity and specificity at trace residue level is required.

Types of Mass analyzer used :

There are a different types of mass analyzers that could be used in LC/MS.

- Single Quadrupole,
- Triple Quadrupole,
- Ion Trap,
- TOF (Time of Flight), and
- Quadrupole-time of flight (Q-TOF).

The quadrupole and ion trap instruments are highly sensitive and hence used for target-oriented residue analysis; whereas the TOF and Q-TOF instruments are mainly used for accurate mass analysis and structure identifications of target and non-target compounds, metabolite identifications, etc.

Applications of Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS is routinely used for detection of :

1) Mycotoxins: LC-MS is routinels used for detection of: Toxins produced by different fungi, e.g. *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., etc. Some of the mycotoxins regularly analyzed in food samples include Aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A, etc.
2) Residual drugs and antibiotics in different food products.
3) Banned dyes and colourants e.g. Sudan dyes in different food products.
4) Residual pesticides in raw and processed food products.

13.8 SPECTROSCOPIC TECHNIQUES

Spectroscopy pertains to the dispersion of an object's light into its component colors (i.e. energies) via prism or the study of the interactions between radiation and matter as a function of wavelength (λ). This technique utilizes interactions between electromagnetic radiation and matter to provide information about food properties, *e.g.,* molecular composition, structure, dynamics and interactions. A variety of the instruments that are commonly used to analyze food materials based on spectroscopy includes, UV-visible, fluorescence, atomic, infrared and nuclear magnetic resonance spectrometer.

13.8.1 Distribution of Energy in Atoms and Molecules

Atoms and molecules can only exist in a limited number of discrete energy levels: they cannot have energies between these levels, *i.e.,* their energy levels are **quantized**. Each molecular species has a unique set of energy levels that depends on its unique atomic structure (electrons, protons, neutrons) and molecular structure (type and arrangement of atoms and bonds). The lowest of these energy levels is referred to as the ground state, while higher levels are referred to as *excited states.* The *potential energy* of an atom or molecule is usually defined relative to the ground state (which is arbitrarily taken to have zero energy). The potential energy of a molecule is made-up of contributions from a number of different sources: electronic, vibrational, rotational, translation and nuclear.

- **Electronic Energy Levels:** Electrons in an atom are arranged into a number of different shells and sub-shells. An electron can move from one of these sub-shell levels to another by absorbing or emitting radiation of appropriate energy. The system is then said to have undergone an *electronic transition.* Electronic transitions may involve electrons that are in inner shells (higher energy) or outer shells (lower energy) of atoms.
- **Vibrational Energy Levels:** Molecules (but not atoms) can vibrate in a number of different modes, *e.g.,* the atoms can compress or stretch along the axis of a bond, or they can bend symmetrically or asymmetrically. Each of these vibrations occurs at a characteristic frequency (energy) that depends on the mass of the atoms and the strength of the bonds involved.

- **Rotational Energy Levels:** Molecules often contain chemical groups that are capable of rotating around certain bonds at fixed frequencies (and therefore energies). Each group has a specific number of frequencies at which it rotates and therefore has a specific number of quantized rotational energy levels. The rotation frequency is determined by the mass of the atoms involved and their distance from the axis of rotation.

- **Nuclear Energy Levels:** The nuclei of certain atoms have a property known as *spin.* A (charged) spinning nucleus generates a small magnetic field and can be thought of as a small magnet. Normally, this magnet can be orientated in any direction, but in the presence of an external magnetic field it can only align itself either with or against the field, *i.e.,* it is quantized. Transitions between the different energy levels within the nuclei can be made to occur by applying radiation of a specific energy to the sample.

- **Translational Energy Levels:** Atoms and molecules are in continual translational motion because of the thermal energy of the system. Translational energy levels are quantized, however, the differences between the energy levels are so small that the molecules act as though the energy is distributed continuously.

13.8.2 Characteristics of Electromagnetic Waves

Electromagnetic waves may be thought of as *small packets* of energy (*photons*) that move through space with *wave*-like properties, *i.e.,* they exhibit *wave-particle* duality (e.g. photoelectric effect). They consist of oscillating electric and magnetic fields that are perpendicular to one another, and to the direction of propagation. The sinusoidal variation in the amplitude of the electric vector of the wave can be plotted as a function of time (at a fixed position within a material) or as a function of distance (at a fixed point in time). A monochromatic (single wavelength) electromagnetic wave that propagates through a vacuum can be described completely by its frequency, wavelength and amplitude (or parameters derived from these):

- The *frequency (v)* of a wave is the number of cycles per second (Hz = s^{-1}).
- The *period (T)* of a wave is the time taken to complete a cycle: $T = 1/v$.
- The *wavelength* λ is the distance between successive maxima/minima of a wave.
- The *wave number* (cm^{-1}) is the number of cycles per unit distance.
- The *amplitude* (*A*) of a wave is the maximum magnitude of the electric vector.
- The *intensity* (*I*) of a wave is proportional to the square of the amplitude. It is the amount of energy passing through a given area per second. Increasing the intensity of an electromagnetic wave increases the *number* of quanta passing a given area per second, not the energy of each individual quantum.
- The *velocity* (*c*) of an electromagnetic wave is the distance travelled per second: $c = \nu \tilde{\lambda}$ The velocity of an electromagnetic wave travelling through a vacuum is the speed of light $c = 3 \times 10^8$ ms^{-1}. The velocity of an electromagnetic wave traveling through a material is always less than that in a vacuum. The refractive index of a material is equal to $c_{vacuum}/c_{material}$.

- The energy (E) of the photons in an electromagnetic wave is related to the frequency of the wave:

  $E = h\nu = h/T = hc/\lambda$

  where, h = Planks constant (6.6262×10^{-34} J s). These expressions can be used to relate the energy of an electromagnetic wave to its frequency, period, wavelength or wave number. This relationship indicates that monochromatic radiation (*i.e.,* radiation of a single frequency) contains photons that all have the same energy.

  The *electromagnetic spectrum* consists of radiation that ranges in wavelength from 10^{-12} m (high energy) to 10^{4} m (low energy). The physical principles and mathematical description of radiation across the whole of the electromagnetic spectrum is the same, however, it is convenient to divide it into a number of different regions depending on the origin of the waves, *i.e.,* cosmic rays, gamma rays, x-rays, ultraviolet, visible, infrared, microwaves, and radio waves.

13.8.3 Interaction of Radiation with Matter

Spectroscopic techniques utilize the fact that atoms and molecules have a discrete set of energy levels and that transitions can only occur between them. When an electromagnetic wave propagates through a material, the atoms or molecules can absorb energy and move to an excited state if the photons in the wave have energies that are exactly equal to the difference between two energy levels ($\Delta E = h\nu$). Alternatively, if an excited atom or molecule emits energy in the form of radiation the waves emitted must have energies that are exactly equal to the difference between two energy levels ($\Delta E = h\nu$). The energy of the photons in different regions of the electromagnetic spectrum corresponds to different types of energetic transitions that can occur in atoms and molecules, *e.g.,* electronic, rotational, vibrational, translational, nuclear transitions. Electromagnetic radiation can therefore be used to probe different molecular characteristics of matter. The atomic or molecular origin of the transitions that occur between different energy levels in matter, the region of the electromagnetic spectrum that these transitions correspond to, and the spectroscopic techniques that can be used to measure these transitions are summarized below:

Transition	**Region of e/m Spectrum**	**Spectroscopy Technique**
Electronic (ĩıkJ mol^{-1})	UV-Visible	UV, Visible, Atomic Fluorescence
Vibrational (10 kJ mol^{-1})	Near and Mid Infrared	Infrared
Rotational (0.1 kJ mol^{-1})	Far Infrared, Microwaves	Infrared
Nuclear (10^{-6} kJ mol^{-1})	Radio Waves	Nuclear Magnetic Resonance (NMR)

The difference between *electronic* energy levels is greater than between *vibrational* energy levels, which is greater than between *rotational* energy levels. Thus higher energy radiation (shorter wavelength) is needed to cause transitions between electronic levels than between vibrational or rotational levels. In practice, a molecule can be thought of as having a number of different electronic energy levels, with rotational and vibrational energy levels superimposed on them.

I) Absorption

Absorption is the process by which energy is transferred from an electromagnetic wave to an atom or molecule and causes it to move to an excited state. Absorption can only occur when an atom or molecule absorbs a photon of light that has energy, which exactly corresponds to the difference between two energy levels, *i.e.,* it must be quantized. At room temperature, the ground state of atoms and molecules is usually the most probable and stable state and hence transitions usually occur from the ground state to higher energy levels. At higher temperatures, more of the higher energy levels are occupied and so, transitions between higher energy levels may also become important.

If an atom or molecule is subjected to electromagnetic radiation of different wavelengths (energies) it will only absorb photons at those wavelengths which correspond to exact differences between two different energy levels within the material. A plot of the fraction of photons absorbed at a particular wavelength versus the energy of the photons at that wavelength is called an *absorption spectrum.* Conventionally, the axis of absorption spectra are specified in terms of easily measurable quantities: *x*-axis shows transmittance or absorbance (rather than fraction of photons absorbed); *y*-axis shows wavelength, frequency or wave number (rather than photon energy).

II) Emission

Emission of radiation is the reverse of absorption, occurring when energy from an atom or molecule is released in the form of a photon of radiation. When a molecule is raised to an excited state it will only exist in this state for a very short time before relaxing back to the ground state. This is because it will always try to move to its lowest energy state. There are two important relaxation processes through which an excited molecule can dissipate its energy:

- **Non-radiative decay:** This is the most common way that an excited molecule loses its energy. Energy is dissipated in a number of small (quantized) steps due to transfer of energy from the excited molecule to surrounding molecules in the form of kinetic energy (heat). Nevertheless, the heat generated is usually so small that it has little effect on the overall temperature of the system.
- **Radiative decay:** In some cases, an atom or molecule loses its energy in the form of a photon (emission). This is the case in atomic emission spectroscopy.

Sometimes both of these processes occur together. In fluorescence spectroscopy, a molecule absorbs electromagnetic radiation, which causes it to move into an excited state. It then returns to the ground state by dissipating some of its energy in the form of non-radiative decay and the rest in the form of a photon of radiation. The photon emitted is therefore of lower energy

(longer wavelength) than the incident wave. Usually, an electron decays to the lowest energy level in the excited electronic state, and then returns to the ground state.

13.8.4 Measurement Modes

The design of an analytical instrument based on spectroscopy depends on the nature of the energetic transitions involved (*e.g.,* electronic, vibration, rotation, translation, nuclear), the nature of the radiative process involved (*e.g.,* absorption, emission, fluorescence) and the nature of the food matrix (*e.g.,* absorbing or non-absorbing). These factors determine the wavelength (frequency) of electromagnetic radiation used, the way that the electromagnetic radiation is generated and the way that the electromagnetic radiation is detected. Some commonly used designs are highlighted below:

- ***Emission***: The sample being analyzed is energetically stimulated (*e.g.,* by heating or application of radiation) and the amount of electromagnetic radiation produced by the sample is measured at different wavelengths, *e.g.,* atomic emission spectroscopy, NMR, fluorescence.
- ***Transmission***: An electromagnetic wave generated by the analytical instrument is propagated directly through the sample and the reduction in its amplitude due to interaction with the sample is measured at different wavelengths, *e.g.,* atomic absorption spectroscopy, infrared transmission measurements, UV-visible spectrophotometery.
- ***Reflection:*** An electromagnetic wave generated by the analytical instrument is reflected from the surface of the sample and the reduction in its amplitude due to interaction with the sample is measured at different wavelengths, *e.g.,* infrared reflection measurements, color measurements.

13.9 SPECTROSCOPIC INSTRUMENTS

The most commonly used spectroscopic instruments for food analysis are:

- UV- Visible Spectroscopy
- Atomic-Absorption Spectroscopy (AAS)
- Inductively Coupled Plasma (ICP)
- Nuclear Magnetic Resonance Spectroscopy (NMR)
- Fourier Transform Infrared Spectroscopy (FTIR)

13.9.1 UV-Visible Spectroscopy

UV-visible spectroscopy is an important tool for the chemical profiling of food products after extraction of the components in the suitable solvent. Chemical profiling using W-visible spectroscopy complemented with chemical profiling with other chromatographic technique can be used as reference for quality control of food products.

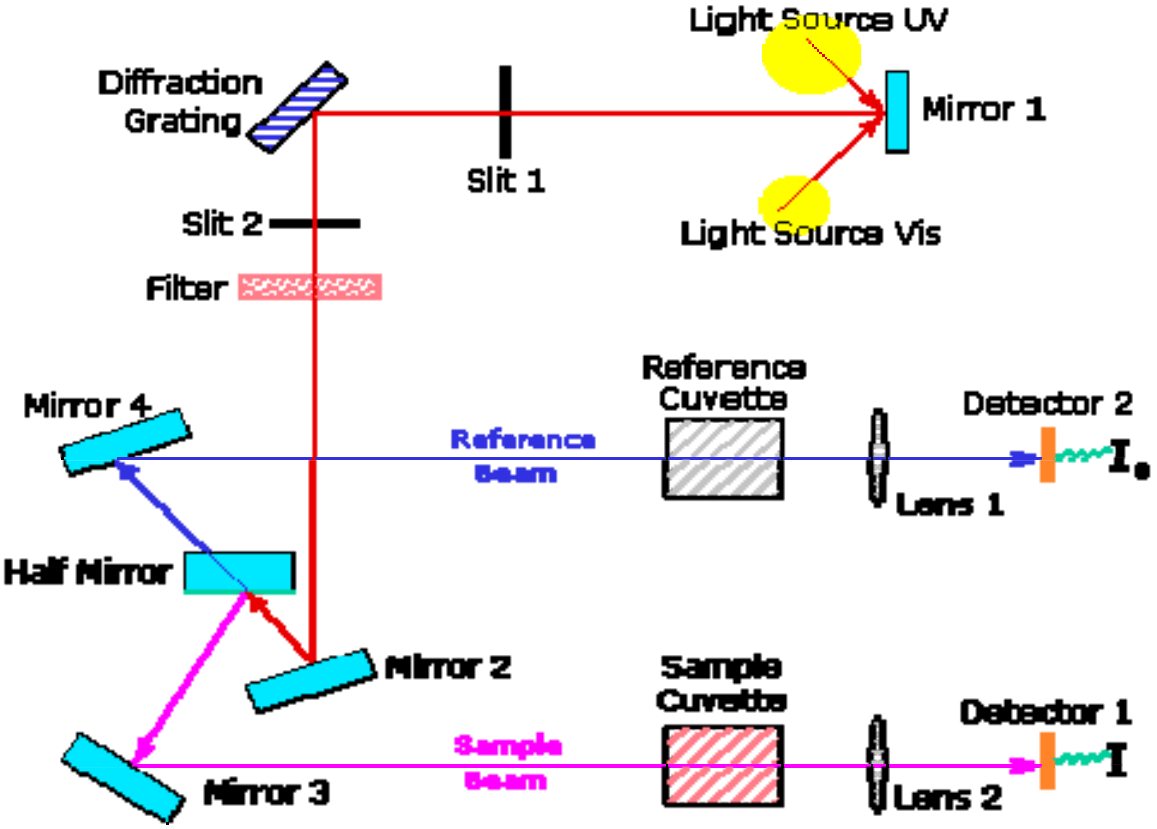

Fig. 13.5: Schematic Diagram of UV-visible Spectroscopy

A beam of light from a visible and/or UV light source is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as $\mathbf{I_0}$. The intensity of the sample beam is defined as **I**. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm. If the sample compound does not absorb light of a given wavelength, $\mathbf{I = I_0}$. However, if the sample compound absorbs light then **I** is less than $\mathbf{I_0}$, Absorption may be presented as **transmittance** ($\mathbf{T = I/I_0}$) or **absorbance** ($\mathbf{A}$= log $\mathbf{I_0/I}$). If no absorption has occurred, T = 1.0 and A= 0. Most spectrometers display absorbance on the vertical axis, and the commonly observed range is from 0 (100% transmittance) to 2 (1% transmittance). The wavelength of maximum absorbance is a characteristic value, designated as λ_{max}. Different compounds may have very different absorption maxima and absorbances.

The most commonly used solvents are water, ethanol, hexane and cyclohexane. Solvents having double or triple bonds, or heavy atoms are generally avoided.

13.9.2 Atomic Absorption Spectroscopy (AAS)

Atomic absorption spectrometry is a very popular method for assessing the concentration of metals and minerals that may be present in the food products. This technique allows measuring all the elements of periodic table. It encompasses a wide variety of techniques and provides rapid, sensitive and selective determination of elemental composition.

Atomic absorption spectrometer has five basic components, which are:

1) A light source (cathode lamp)
2) A sample cell (absorption cell)
3) Monochromator
4) Detector
5) Output unit

In this technique, the elements in the sample are brought into their ionized form in solution by using the wet digestion, dry ashing or suitable microwave assisted digestion system and then aspirated through a nebulizer into the high temperature flame where the sample gets converted into gaseous atoms. The source of light is usually a hollow cathode lamp, which is composed of the element being measured. Each element requires a different lamp. The hollow cathode lamp produces emission lines specific for the element used to construct the cathode. The lamp is filled with an inert gas like argon or neon. When a potential is applied, the gas is ionized and is driven towards the cathode and cause the metal atoms to sputter all the surface of the cathode and produce specific atomic emission lines.

The characteristic emission lines produced by the source i.e. hollow cathode lamp are absorbed by the atoms which get excited and are raised to higher energy level. As the sample passes through the flame, the beam of light passes through the monochromator. The monochromator isolates the specific spectrum line emitted by the light source through spectral dispersion and focuses it upon a photo multiplier detector where light signal is converted into an electrical signal. The process of electrical signal is fulfilled by a signal amplifier. The signal could be displayed for readout or further fed into a data station.

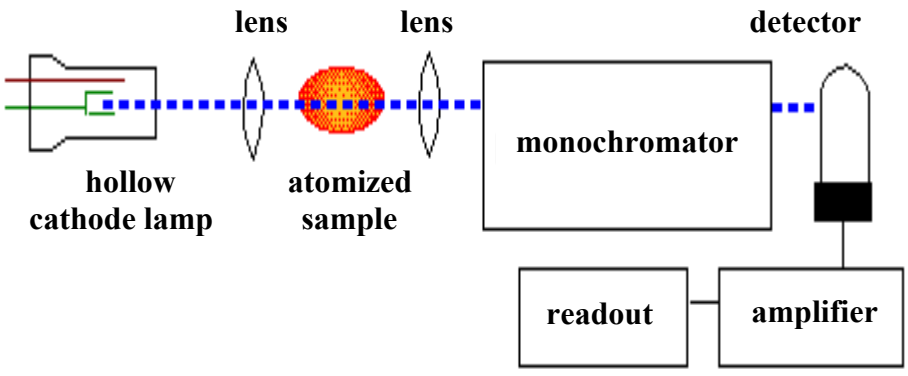

Fig. 13.6: Schematic of an Atomic-Absorption Experiment

The greater amount of sample present, the higher the absorbance energy. Different flames can be achieved by using different mixtures of gases,

depending on the desired temperature and burning velocity. Some elements can only be converted to atoms at high temperatures. Even at high temperatures, if excess oxygen is present, some metals form oxides that do not re-dissociate into atoms. To inhibit their formation, conditions of the flame may be modified to achieve a reducing, non-oxidizing flame. The most widely used flames are air-acetylene and nitrous oxide-acetylene flame. The nitrous oxide-acetylene flame has a higher temperature as compared to air-acetylene flame.

A) Electrothermal Atomization

In determination of certain elements (Ag, Be, Ca, Fe, Pb and Sn), flame atomic absorption spectroscopy may not be very sensitive. Use of electro thermal atomization provides sensitivity up to the levels of parts per billion (ppb) to subparts per billion levels. This type of atomization requires a graphite furnace, which is a graphite tub, where the sample is rapidly atomized after thermal pre-treatment. To maintain a dense fraction of free ground state elements in the optical path, an inert gas atmosphere is used. Since the dilution and expansion effects of flame cells are avoided, and the atoms have a longer residence time in the optical path, a higher peak concentration of atoms is obtained.

B) Vapour Generation- Atomic Absorption Spectroscopy (AAS-VGA)

Vapour Generation- Atomic Absorption Spectroscopy (AAS-VGA) is used for the determination of hydride-forming elements, such as As, Hg, Sn, etc., using quartz tube atomizer. VGA-AAS is highly sensitive and effective method for quantification of toxic metals in food products and can be used for the determination of these elements at ppm to ppb levels.

Applications of Atomic-absorption spectroscopy

1) Atomic absorption spectroscopy can be used to analyze the concentration of esssential minerals and toxic metals in all raw and processed food products.

13.9.3 Inductively Coupled Plasma – Optical Emmission Spectrophotometry (ICP-OES/MS)

Inductively Coupled Plasma (ICP) is an analytical technique used for the detection of trace metals in Food samples. The primary goal of ICP is to get elements to emit radiations of characteristic wavelength that can then be measured.

An ICP requires that the elements, which are to be analyzed, in the solution form. An aqueous solution is preferred over an organic solution, as organic solutions require a special accessory prior to introduction of a sample into the ICP. Solid samples are also discouraged, as clogging of the instrument can occur. The nebulizer transforms the aqueous solution into an aerosol. The light emitted by the atoms of an element in the ICP must be converted to an electrical signal that can be measured quantitatively

An ICP typically includes the following components:

- Sample introduction system (nebulizer)
- ICP torch
- High frequency generator

- Transfer optics and spectrometer
- Computer interface

ICP is an atomic emission technique using argon plasma as an excitation source. The sample is introduced into a premix spray chamber, where it is directed up the central tube of the ICP ‘‘torch’’. The torch consists of concentric tubes with independent argon streams flowing through each tube. The top of the torch is centered within a Radio Frequency (RF) induction coil, which is the source of energy for the system. After ignition, the plasma is propagated through inductive coupling with the RF field generated from the coil. The ICP torch is designed specifically to promote penetration of the plasma skin by the sample, allowing sample atoms to experience the full energy of the plasma source. The high temperatures provided by the ICP provide excellent sensitivities for refractory elements and also essentially eliminate chemical interferences. Like all emission techniques, there are no source lamps. By monitoring several wavelengths, either all at once or in a programmed sequence, many elements can be determined in one automated analysis. ICP emission, therefore, offers significant speed advantages over atomic absorption for multi element analysis. Except for the refractory elements, which may be substantially better than even graphite furnace AA, ICP detection limits are comparable to flame atomic absorption. The high temperatures of the ICP carry one disadvantage. The plasma is so effective in generating excited state species that the rich emission spectra produced increase the probability of spectral interferences. High resolution monochromators and sophisticated software for background and inter element correction are used to deal with this potential problem. Another limitation of ICP emission is the initial cost of the instrument. The price for basic ICP systems starts at about the same level as the prices for top-of-the-line automated AA systems. More sophisticated instrument can cost two to four times the price of basic systems.

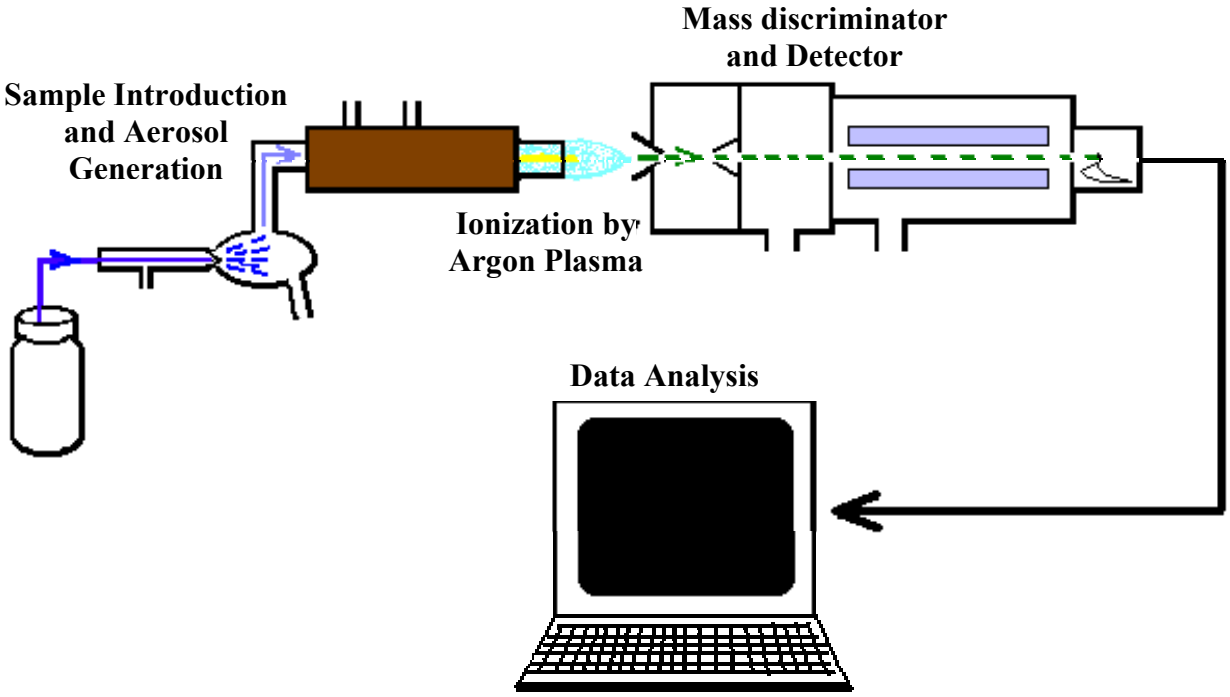

Fig. 13.7: Schematic Diagram of ICP – OES

13.9.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectroscopy exploits the magnetic properties of certain nuclei. The most important applications for the organic chemist are proton NMR and carbon-13 NMR spectroscopy. In principle, NMR is applicable to any nucleus possessing spin.

Much information can be obtained from an NMR spectrum. Analysis of a NMR spectrum provides information on the number and type of chemical entities in a molecule. A bit more detail + enclosures followed.

The impact of NMR spectroscopy on the natural sciences has been substantial. It can, among other things, be used to study mixtures of analytes, to understand dynamic effects such as change in temperature and reaction mechanisms, and is an invaluable tool in understanding protein and nucleic acid structure and function. It can be applied to a wide variety of samples, both in the liquid and the solid state.

13.9.5 Fourier Transform-Infrared Spectroscopy (FT-IR)

The mid-IR region covers the range from 4000 to 400 cm^{-1}. The basic principle of the IR technique is that various organic functional groups absorb infrared light at specific wavelengths. Thus, since every organic molecule has a unique chemical composition, it also has a unique infrared spectrum. Biological samples are composed of proteins, carbohydrates, lipids and nucleic acids. Since these molecules contain functional organic groups, the IR spectrum consists of bands from all these components.

The infrared spectrum is very complex, and it contains a large amount of information. To evaluate the data, it is necessary to use multivariate statistical analysis.

Some of the applications of FT-IR Spectroscopy for food analysis include:

1) Characterization of essential oils in various spices
2) Evaluation of trans fats in oils and fats

Check Your Progress Exercise 2

Note: a) Use the space below for your answers.
b) Check your answers with those given at the end of the unit.

1) What do you understand by Hyphenated techniques? List any two examples of this technique?

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2) Which Spectroscopic technique is used for analyzing the concentration of metals and minerals in food products?

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3) What are the application of FT-IR Spectroscopy for food analysis?

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13.10 THERMAL METHODS OF ANALYSIS

Most foods are subjected to variations in their temperature during production, transport, storage, preparation and consumption, e.g. pasteurization, sterilization, evaporation, cooking, freezing, chilling etc. Temperature changes cause alterations in the physical and chemical properties of food components which influence the overall properties of final product e.g., taste, appearance, texture and stability. Chemical reaction such as hydrolysis, oxidation or reduction may be enhanced, or physical changes, such as evaporation, melting, crystallization, aggregation or gelation may occur. A better understanding of the influence of the temperature on the properties of food enables food manufacturers to optimize processing condition and improve product quality. It is therefore important for food scientist to have analytical technique to monitor the changes that occur in food. These techniques are often grouped under the general heading thermal analysis, at present the term thermal analysis is usually reserved for a narrow range of technique that measures changes in the physical properties of foods with temperature e.g. mass, density, rheology, and heat capacity.

A variety of differential analytical technique have been deployed to monitor changes in the physical properties of food components that occurs in response to controlled changes in temperature. A number of most important of these thermal analysis techniques are described below:

13.10.1 Thermogravimetry

Thermogravimetry technique continuously measure the mass of a sample as it is heated or cooled at a controlled rate, or it is held at a particular temperature for a period of time with reference to a control sample. Thermogravimetry is useful for monitoring process that involves a change in the mass of food or food component, e.g., drying, liberation of gasses, absorption of moisture. To study the various types of processing and storage condition that a food might normally experience, thermogravimetric instruments have been specially designed to allow measurements to be carried out under specific environments e.g., controlled pressures or atmosphere. Gravimetric instruments typically consist of a sensitive balance situated within a container whose pressure, temperature and gaseous environment can be carefully controlled. The ability to carefully control the temperature, pressure and composition of the gases surrounding a sample is extremely valuable for food scientist, because it allows them to model processes such as drying, cooking, and uptake of moisture during storage.

Differential Thermal Analysis and Differential Scanning Calorimetry

DTA and DSC are techniques rely on changes in the heat absorbed or released by a material as its temperature is varied at a controlled rate. These changes occur when components within a food undergo some type of phase transition

(e.g., crystallization, melting, evaporation, glass transitions, conformational change) or a chemical reaction (e.g. oxidation, hydrolysis)

13.10.2 Differential Thermal Analysis (DTA)

DTA is defined as a technique for recording the difference in temperature between a substance and a reference material against time or temperature as the two specimen are subjected to identical temperature regimes in a environment heated or cooled at a controlled rate. A typical instrument consists of two measurement cells that are located in a temperature controlled environment, whose temperature can be varied in a programmed manner. The sample to be tested is placed into the 'sample cell' while a reference material of known thermal properties (often distilled water) is placed in the "reference cell". The two cells are then heated or cooled at a controlled rate. The small difference in temperature between a "sample cell" and "reference cell" ($\Delta T = T_{sample} - T_{reference}$) is measured using accurate thermocouples placed below the cells as the temperature of the external environment ($T_{external}$) is varied in a controlled fashion. The output of the instrument is therefore a plot of ΔT versus $T_{external}$. The position of the peak provides information about the temperature that the transition occurs. The under a peak depends on the amount of material involved in the transition and the enthalpy change per unit amount of material.

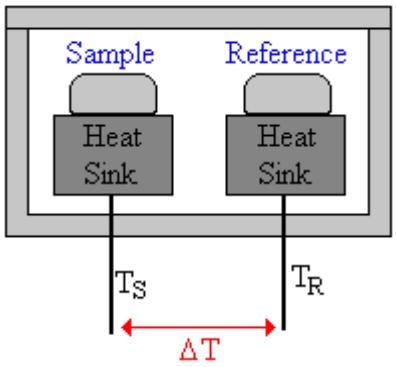

Fig. 13.8: Differential Thermal Analysis

13.10.3 Differential Scanning Calorimetry (DSC)

DSC is a technique for recording the energy required to keep a zero temperature difference between a sample cell and a reference cell that are heated or cooled at a controlled rate. The thermocouples constantly measure the temperature of each cell and heaters supply heat to one or other of the cells so that they both have exactly the same temperature. If a sample were to undergo a phase transition it would either absorb or release heat. DSC data is therefore reported as the rate of energy absorption (Q) by the sample relative to the reference material as a function of external temperature. Information about thermal transitions that occur within a sample is obtained by analyzing the Q versus T external thermo gram. It should be noted that it is possible to measure the change in the heat release by a material as a function of time under isothermal (constant temperature) conditions.

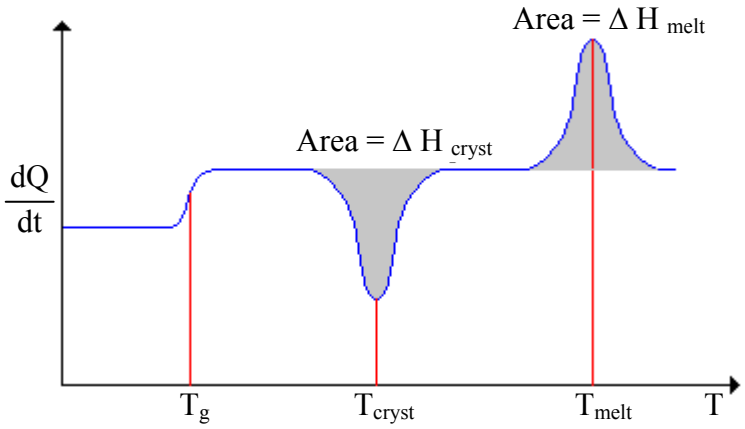

Fig. 13.9: An illustrated Thermogram

Applications

- **Determining Specific heat capacity:** The Specific heat capacity is an important physical quantity in food industry, since it is the amount of energy that must be supplied or withdrawn from a material in order to increase or decrease its temperature by a 1ºC. The knowledge of Specific heat capacity of a material is therefore important in the design of processes such as chilling, freezing, warming, sterilization and cooking.

- **Phase transitions:** DSC and DTA are routinely used in the food industry to characterize phase transitions in foods, for e.g. crystallization, melting, glass transitions and conformational changes. They can be used to provide information about the temperature at which transitions occur. When a material changes its physical state from solid to liquid (melting) or from liquid to solid (crystallization) it absorbs or gives out heat respectively. Pure substances usually have very sharp melting or crystallization points and therefore all the heat is absorbed or evolved over a narrow range of temperatures, leading to sharp DSC or DTA peak. Many food components are chemically complex materials and therefore the phase transitions occur over a wide range of temperature, e.g. edible oils contain a wide variety of different triacylglycerols each with its own melting points. Peaks from food oils may also be complicated by the fact that triacylglycerols can crystallize in more than one different crystalline structure i.e. they are polymorphic.

Check Your Progress Exercise 3

Note: a) Use the space below for your answers.
b) Check your answers with those given at the end of the unit.

1) Name different types of Thermal analysis methods used in food?

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2) Describe briefly the various applications of Thermal method of analysis in food?

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13.11 KEY WORDS

Chromatography : It is a technique where solutes are separated by a dynamic differential migration in a system consisting of two or more phases where one is mobile phase and the other is a stationary phase.

Spectroscopy : Spectroscopy pertains to the dispersion of a light into its component colors (i.e. energies) via prism or the study of the interaction between radiation and matter as a function of wavelength (λ).

Mass Spectrometry : Mass spectrometry is an analytical technique that identifies the identity of a chemical substance on the basis of the mass to charge ratio of fragmented charged particles.

HPLC : It is a separation technique with a solid stationary phase (column) and a liquid mobile phase.

GC : It is a separation technique with a solid stationary phase (column) and gaseous mobile phase. Generally used for volatile compounds analysis.

AAS : Atomic spectroscopy is the study of absorption and emission characterictics of geseous atoms of elements.

ICP : Elements are excited by Argon plasma to gaseous ions which are further identified and quantified by emission spectroscopy or mass spectrometry.

Rheological Technique : It is the study of deformation and flow in food or material in response to an applied load or deformation.

Hyphenated Technique : It is the combination of two or more analytical techniques, e.g. chromatographic separation system (HPLC/GC) on-line with a spectroscopic detector (MS), in order to obtain structural information of the analytes present in a sample.

NMR Spectroscopy : NMR spectroscopy is the technique of studying structural properties of compounds such as type and nature of bonds using the magnetic properties of certain nuclei possessing spin.

Wheatstone Bridge : It is an electrical circuit. In wheat-stone bridge four resistance R_1, R_2, R_3 and R_4 are connected end to end with each other to form a closed loop. A sensitive glavanometer "G" is connected between their junctions. The circuit is provided with two keys "K_1" and "K_2". Generally wheat-stone bridge is used to determine unknown resistances.

13.12 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include following points:

1) It is a technique where solutes are separated by a dynamic differential migration in a system, consisting of two or more phases where one is mobile phase and the other is a stationary phase.

2) Gas Chromatography can be applied for analysis and determination of different compounds in food products such as:

   a) Cholesterol, Fatty acid profiling and Trans fat analysis;

   b) Antioxidants and Preservatives like TBHQ, Benzoic acid, Sorbic acid Acetic acid, etc;

   c) Analysis of residual pesticides and environmental contaminants;

   d) Characterization of flavours and fragrances; and

   e) The Gas Chromatographic profiling of the essential volatile oils gives a reasonable 'fingerprint' which can be used to characterize the identity of the particular oil.

3) **Detector used for gas chromatography are:**

   Flame Ionisation Detector (FID), Electron Capture Detector (ECD),

   Nitrogen Phosphorous Detector (NPD) Thermal Conductivity Detector (TCD)

   Detector used for HPLC are:

   Ultra Violet (UV), Photo Diode Array (PDA), Refractive Index (RI), Fluorescent, and Mass Spectrometric (MS) detector.

Check Your Progress Exercise 2

Your answer should include following points:

1) It is the combination of two or more analytical techniques, e.g. chromatographic separation system (HPLC/GC) on-line with a spectroscopic detector (MS), in order to obtain structural information of the analytes present in a sample.

   Examples: GC-MS, LC-MS

2) Atomic Absorption Spectroscopy

3) Applications of FT-IR Spectroscopy for food analysis include:

   a) Characterization of essential oils in various spices.

   b) Evaluation of trans fats in oils and fats.

Check Your Progress Exercise 3

Your answer should include following points:

1) Thermogravimetry

   Differential Thermal Analysis

   Differential Scanning Calorimetry (DSC)

2) a) Thermal method of analysis is useful in measuring specific heat capacity of a material.

   b) DSC and DTA are used to characterise phase transition in foods e.g. crystallization, melting, glass transition and conformational changes.

13.13 SOME USEFUL BOOKS

S.S. Nielsen, (1998). *Introduction to Food Analysis*. Aspen Publishers - The best general overview of food analysis techniques currently available. .

Y. Pomeranz and C.E. Meloan. *Food Analysis: Theory and Practice*. Chapman and Hall - General overview of food analysis techniques.

D.W. Gruenwedel and J.R. Whitaker. *Food Analysis: Principles and Techniques*. Marcel Dekker - General overview of food analysis techniques.

C.S. James. *Analytical Chemistry of Foods*. Blackie Academic and Professional - General overview of food analysis techniques.

Official Methods of Analysis, Association of Official Analytical Chemists - Officially recognized methods of analysis for many food components.