R.M. Akhmadullin, R.R. Spiridonova, A.M. Kochnev

BASIC AND APPLIED POLYMER RESEARCH METHODS

Tutorial

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Основы экспериментальных методов исследования в области полимеров: Учебное пособие / Р.М. Ахмадуллин, Р.Р. Спиридонова, А.М. Кочнев; Федер. Агенство по образованию, Казан. нац. исслед. технол. ун-т. – Казань: КНИТУ, 2012. – 75с.

Рассмотрены основные методы исследования полимеров, применяемые в отечественной и зарубежной практике. Среди них ядерный магнитный резонанс, электронный парамагнитный резонанс; инфракрасная, рентгеновская, УФ и видимая спектроскопии; хроматография, масс-спектрометрия, термогравиаметрические методы анализа, калориметрия, дилатометрия; оценка стойкости полимеров к внешним воздействиям и эффективности действия стабилизаторов.

Учебное пособие предназначено для студентов 5 курса полимерного факультета обучающихся по направлению подготовки 020015 «Химическая технология», знакомых с основными понятиями и законами химии и физики высокомолекулярных соединений, методами их синтеза, кинетическими и термодинамическими закономерностями полимеризации и поликонденсации, фазовыми и физическими состояниями полимеров. Изложенный в учебном пособии материал ознакомит студентов со спецификой физико-химических методов анализа применительно к современным полимерным материалам, а выполнение практических работ на современном оборудовании поможет получить будущим дипломированным специалистам навыки работы для дальнейшего применения в научных и производственных лабораториях.

Содержание учебного пособия соответствует программе курса «Основы экспериментальных методов исследования в области полимеров».

Рецензенты:

Доктор химических наук,
Профессор кафедры высокомолекулярных и
элементорганических соединений КФУ И.В. Галкина

Кандидат химических наук, научный сотрудник
лаборатории элементорганического синтеза
ИОФХ им. А.Е. Арбузова КазНЦ РАН А.С. Газизов

CONTENT

1. ESSENCE OF RESEARCH METHODS

Successful characterization of polymer systems is one of the most important objectives of today's experimental research of polymers. Considering the tremendous scientific, technological, and economic importance of polymeric materials, not only for today's applications but for the industry of the 21st century, it is impossible to overestimate the usefulness of experimental techniques in this field. Since the chemical, pharmaceutical, medical, and agricultural industries, as well as many others, depend on this progress to an enormous degree, it is critical to be as efficient, precise, and cost-effective in our empirical understanding of the performance of polymer systems as possible. This presupposes our proficiency with, and understanding of, the most widely used experimental methods and techniques.

1.1 Concepts and Definitions

Scientific method refers to a body of techniques for investigating phenomena, acquiring new knowledge, or correcting and integrating previous knowledge.

Research can be defined as the search for knowledge, or as any systematic investigation, with an open mind, to establish novel facts, solve new or existing problems, prove new ideas, or develop new theories.

Structure is a fundamental, tangible or intangible notion referring to the recognition, observation, nature, and permanence of patterns and relationships of entities.

Property is a philosophical category expressing the attitude of the thing to other things it interacts with.

Polymer is any of various chemical compounds made of smaller, identical molecules (called monomers) linked together.

1.2 Scientific Research

Generally, research is understood to follow a certain structural process. Though step order may vary depending on the subject matter and researcher, the following steps are usually part of most formal research, both basic and applied:

1. Observations and formation of the topic: Consists of the subject area of one's interest and following that subject area to conduct subject related research. The subject area should not be randomly chosen since it requires reading a vast amount of literature on the topic to determine the gap in the literature the researcher intends to narrow. A keen interest in the chosen subject area is advisable. The research will have to be justified by linking its importance to already existing knowledge about the topic.
2. Hypothesis: A testable prediction which designates the relationship between two or more variables.
3. Conceptual definition: Description of a concept by relating it to other concepts.
4. Operational definition: Details in regards to defining the variables and how they will be measured/assessed in the study.
5. Gathering of data: Consists of identifying a population and selecting samples, gathering information from and/or about these samples by using specific research instruments. The instruments used for data collection must be valid and reliable.
6. Analysis of data: Involves breaking down the individual pieces of data in order to draw conclusions about it.
7. Data Interpretation: This can be represented through tables, figures and pictures, and then described in words.
8. Test, revising of hypothesis.
9. Conclusion, reiteration if necessary.

A common misconception is that a hypothesis will be proven. Generally a hypothesis is used to make predictions that can be tested by observing the outcome of an experiment. If the outcome is inconsistent with the hypothesis, then the hypothesis is rejected. However, if the outcome is consistent with the hypothesis, the experiment is said to support the hypothesis. This careful language is used because researchers recognize that alternative hypotheses may also be consistent with the observations. In this sense, a hypothesis can never be proven, but rather only supported by surviving rounds of scientific testing and, eventually, becoming widely thought of as true.

A useful hypothesis allows prediction and within the accuracy of observation of the time, the prediction will be verified. As the accuracy of observation improves with time, the hypothesis may no longer provide an accurate prediction. In this case a new hypothesis will arise to challenge the old, and to the extent that the new hypothesis makes more accurate predictions than the old, the new will supplant it. Researchers can also use a null hypothesis, which state no relationship or difference between the independent or dependent variables. A null hypothesis uses a sample of all possible people to make a conclusion about the population.

1.3 Scientific Research Methods

The goal of the research process is to produce new knowledge, or deepen understanding of a topic or issue. This process takes three main forms:

- Exploratory research, which structures and identifies new problems.
- Constructive research, which develops solutions to a problem.
- Empirical research, which tests the feasibility of a solution using empirical evidence.

Research can also fall into two distinct types:

- Primary research: Original findings.
- Secondary research: Summary, collation and/or synthesis of existing research.

Scientific research is a robust and dynamic practice that employs multiple methods toward investigating phenomena, including experimentation, description, comparison, and modeling. Though these methods are described separately both here and in more detail in subsequent modules, many of these methods overlap or are used in combination. For example, when NASA scientists purposefully slammed a 370 kg spacecraft named Deep Impact into a passing comet in 2005, the study had some aspects of descriptive research and some aspects of experimental research. Many scientific investigations largely employ one method, but different methods may be combined in a single study, or a single study may have characteristics of more than one method. The choice of which research method to use is personal and depends on the experiences of the scientists conducting the research and the nature of the question they are seeking to address. Despite the overlap and interconnectedness of these research methods, it is useful to discuss them separately to understand the principal characteristics of each and the ways they can be used to investigate a question.

Experimentation: Experimental methods are used to investigate the relationship(s) between two or more variables when at least one of those variables can be intentionally controlled or manipulated. The resulting effect of that manipulation (often called a treatment) can then be measured on another variable or variables. The work of the French scientist Louis Pasteur is a classic example. Pasteur put soup broth in a series of flasks, some open to the atmosphere and others sealed. He then measured

the effect that the flask type had on the appearance of microorganisms in the soup broth in an effort to study the source of those microorganisms.

Description: Description is used to gather data regarding natural phenomena and natural relationships and includes observations and measurements of behaviors. A classic example of a descriptive study is Copernicus's observations and sketches of the movement of planets in the sky in an effort to determine if the Earth or the Sun is the orbital center of those objects.

Comparison: Comparison is used to determine and quantify relationships between two or more variables by observing different groups that either by choice or circumstance are exposed to different treatments. Examples of comparative research are the studies that were initiated in the 1950s to investigate the relationship between cigarette smoking and lung cancer in which scientists compared individuals who had chosen to smoke of their own accord with non-smokers and correlated the decision to smoke (the treatment) with various health problems including lung cancer.

Modeling: Both physical and computer-based models are built to mimic natural systems and then used to conduct experiments or make observations. Weather forecasts are an example of scientific modeling that we see every day, where data collected on temperature, wind speed, and direction are used in combination with known physics of atmospheric circulation to predict the path of storms and other weather patterns.

These methods are interconnected and are often used in combination to fully understand complex phenomenon. Modeling and experimentation are ways of simplifying systems towards understanding causality and future events. However, both rely on assumptions and knowledge of existing systems that can be provided by descriptive studies or other experiments. Description and comparison are used to understand existing systems and are used to examine the application of experimental and modeling results in real-world systems. Results from descriptive and comparative studies are often used to confirm causal relationships identified by models and experiments. While some questions lend themselves to one or another strategy due to the scope or nature of the problem under investigation, most areas of scientific research employ all of these methods as a means of complementing one another towards clarifying a specific hypothesis, theory, or idea in science. Scientific theories are clarified and strengthened through the collection of data from more than one method that generate multiple lines of evidence.

2. CHROMATOGRAPHY METHODS

2.1 Basic Concepts, Terminology

Chromatography (from Greek χρῶμα *chroma* "color" and γράφειν *graphein* "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

The **stationary phase** is the substance which is fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography.

The **mobile phase** is the phase which moves in a definite direction. It may be a liquid (LC and Capillary Electrochromatography (CEC)), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated/analyzed and the solvent that moves the sample through the column. In the case of High-performance liquid chromatography (or HPLC) the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromotagraphy and the sample being separated. The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.

Adsorbent is a solid sorbent, concentrating on its surface dissolved substances.

Absorbent is the solid or liquid sorbent that dissolving gases, vapors or liquid components of mixtures.

The **eluant** is the liquid used as a mobile phase.

The **eluate** is the mobile phase leaving the column.

The **solute** refers to the sample components in partition chromatography.

The **solvent** refers to any substance capable of solubilizing other substance, and especially the liquid mobile phase in LC.

The **sample** is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.

The **injector** (dispenser) is the part of the chromatograph that designed for the sample input.

The **column** is a tube filled with sorbent or hollow tube coated by sorbent, where the chromatographic separation of the mixture is carried out.

The **detector** is the part of the chromatograph, which is used to convert changes of the physical or physico-chemical parameters of the mobile phase into the electric signal sent to the registrar of the chromatogram.

A **chromatogram** (see Fig.1) is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture. Plotted on the x-axis is the retention time and plotted on the y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

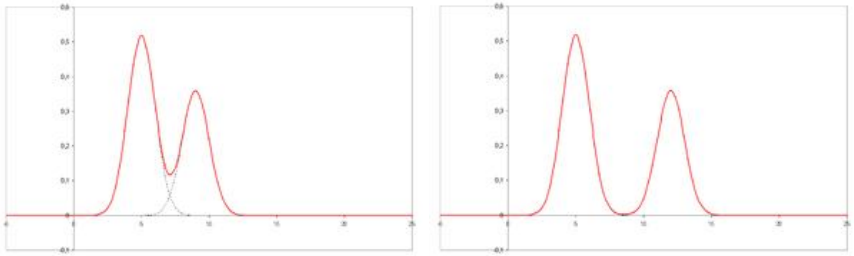


Fig.1 Chromatogram.

The **retention volume** is the volume of mobile phase passed through the column between the injection point and the peak maximum.

The **retention time** is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.

2.2 Chromatography Techniques

2.2.1 Chromatographic Bed Shape

Column Chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

In 1978, W. C. Still introduced a modified version of column chromatography called flash column chromatography (flash). The technique is very similar to the traditional column chromatography, except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage.

In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.

Planar Chromatography

Planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (paper chromatography) or a layer of solid particles spread on a support such as a glass plate (thin layer chromatography). Different compounds in the sample mixture travel different distances according to how strongly they interact with

the stationary phase as compared to the mobile phase. The specific Retention factor (R_f) of each chemical can be used to aid in the identification of an unknown substance.

Paper Chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of chromatography paper. The paper is placed in a jar containing a shallow layer of solventand sealed. As the solvent rises through the paper, it meets the sample mixture which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

Thin Layer Chromatography

Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification, high-performance TLC can be used.

Displacement Chromatography

The basic principle of displacement chromatography is: a molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities. There are distinct differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational

complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than “peaks”. Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

2.2.2 Physical State of Mobile Phase

Gas Chromatography

Gas chromatography (GC), also sometimes known as Gas-Liquid Chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatography is always carried out in a column, which is typically "packed" or "capillary".

Gas chromatography is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium). The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat will denature them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring and remediation, and industrial chemical fields. It is also used extensively in chemistry research.

Liquid Chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC).

In HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Methods in which the stationary phase is more polar than the mobile phase (e.g. toluene as the mobile phase, silica as the stationary phase) are termed

normal phase liquid chromatography (NPLC) and the opposite (e.g. water-methanol mixture as the mobile phase and C18 = octadecylsilyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC). Ironically the "normal phase" has fewer applications and RPLC is therefore used considerably more.

Specific techniques which come under this broad heading are listed below. It should also be noted that the following techniques can also be considered fast protein liquid chromatography if no pressure is used to drive the mobile phase through the stationary phase.

Supercritical Fluid Chromatography

Supercritical fluid chromatography is a separation technique in which the mobile phase is a fluid above and relatively close to its critical temperature and pressure.

2.2.3 Separation Mechanism

Ion Exchange Chromatography

Ion exchange chromatography (usually referred to as ion chromatography) uses an ion exchange mechanism to separate analytes based on their respective charges. It is usually performed in columns but can also be useful in planar mode. Ion exchange chromatography uses a charged stationary phase to separate charged compounds including anions, cations, amino acids, peptides, and proteins. In conventional methods the stationary phase is an ion exchange resin that carries charged functional groups which interact with oppositely charged groups of the compound to be retained. Ion exchange chromatography is commonly used to purify proteins using FPLC.

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) is also known as gel permeation chromatography (GPC) or gel filtration chromatography and separates molecules according to their size (or more accurately according to their hydrodynamic diameter or hydrodynamic volume). Smaller molecules are able to enter the pores of the media and, therefore, molecules are trapped and removed from the flow of the mobile phase. The average residence time in the pores depends upon the effective size of the analyte molecules. However, molecules that are larger than the average pore size of the packing are excluded and thus suffer essentially no retention; such species are the first

to be eluted. It is generally a low-resolution chromatography technique and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins, especially since it can be carried out under native solution conditions.

2.2.4 Special Techniques

Reversed-Phase Chromatography

Reversed-phase chromatography is an elution procedure used in liquid chromatography in which the mobile phase is significantly more polar than the stationary phase.

Two-Dimensional Chromatography

In some cases, the chemistry within a given column can be insufficient to separate some analytes. It is possible to direct a series of unresolved peaks onto a second column with different physico-chemical (Chemical classification) properties. Since the mechanism of retention on this new solid support is different from the first dimensional separation, it can be possible to separate compounds that are indistinguishable by one-dimensional chromatography. The sample is spotted at one corner of a square plate, developed, air-dried, then rotated by 90° and usually redeveloped in a second solvent system.

Simulated Moving-Bed Chromatography

In chromatography, the simulated moving bed (SMB) technique is a variation of high performance liquid chromatography; it is used to separate particles and/or chemical compounds that would be difficult or impossible to resolve otherwise. This increased separation is brought about by a valve-and-column arrangement that is used to lengthen the stationary phase indefinitely.

Pyrolysis Gas Chromatography

Pyrolysis gas chromatography mass spectrometry is a method of chemical analysis in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry.

Pyrolysis is the thermal decomposition of materials in an inert atmosphere or a vacuum. The sample is put into direct contact with a platinum wire, or placed in a quartz sample tube, and rapidly heated to 600–1000°C. Depending on the application even higher temperatures are used. Three different heating techniques are used in actual pyrolyzers: Isothermal furnace, inductive heating (Curie Point filament), and resistive heating using platinum filaments. Large molecules cleave at their weakest points and produce smaller, more volatile fragments. These fragments can be separated by gas chromatography. Pyrolysis GC chromatograms are typically complex because a wide range of different decomposition products is formed. The data can either be used as fingerprint to prove material identity, or the GC/MS data is used to identify individual fragments to obtain structural information. To increase the volatility of polar fragments, various methylating reagents can be added to a sample before pyrolysis.

Besides the usage of dedicated pyrolyzers, pyrolysis GC of solid and liquid samples can be performed directly inside Programmable Temperature Vaporizer (PTV) injectors that provide quick heating (up to 30°C/s) and high maximum temperatures of 600–650°C. This is sufficient for some pyrolysis applications. The main advantage is that no dedicated instrument has to be purchased and pyrolysis can be performed as part of routine GC analysis. In this case quartz GC inlet liners have to be used. Quantitative data can be acquired, and good results of derivatization inside the PTV injector are published as well.

Pyrolysis gas chromatography is very useful for the identification of synthetic polymeric media, such as acrylics or alkyds, and synthetic varnishes. It can also be used for environmental samples, including fossils. Trace evidence scientists in forensic laboratories use Pyroylsis GC to compare and identify paint or finish found at crime scenes or victims.

Countercurrent Chromatography

Countercurrent chromatography (CCC) is a type of liquid-liquid chromatography, where both the stationary and mobile phases are liquids. The operating principle of CCC equipment requires a column consisting of an open tube coiled around a bobbin. The bobbin is rotated in a double-axis gyratory motion (a cardioid), which causes a variable gravity (G) field to act on the column during each rotation. This motion causes the column to see one partitioning step per revolution and components of the sample separate in the column due to their partitioning coefficient between the two im-

miscible liquid phases used. There are many types of CCC available today. These include HSCCC (High Speed CCC) and HPCCC (High Performance CCC). HPCCC is the latest and best performing version of the instrumentation available currently.

Chiral Chromatography

Chiral chromatography involves the separation of stereoisomers. In the case of enantiomers, these have no chemical or physical differences apart from being three-dimensional mirror images. Conventional chromatography or other separation processes are incapable of separating them. To enable chiral separations to take place, either the mobile phase or the stationary phase must themselves be made chiral, giving differing affinities between the analytes. Chiral chromatography HPLC columns (with a chiral stationary phase) in both normal and reversed phase are commercially available.

2.3 Chromatograph

A chromatograph is an instrument that is used to carry out chromatographic separations. The chromatograph consists of five different entities. The mobile phase supply system, the injection or sampling system, the column and column oven, the detector and the data acquisition and data processing system. In gas chromatography the mobile phase supply system consists of gas tanks, reducing valves, flow controllers and pressure gauges. In liquid chromatography the mobile phase supply system consists of a set of solvent reservoirs, sparged with helium gas to remove dissolved air, a solvent selector valve, a solvent programmer and a high pressure pump. In gas chromatography, the sampling system, in its simplest form, consists of a septum injector and a sampling syringe. In its more sophisticated form, it will include an automatic sampling device that may also be under the chromatograph computer control. Liquid chromatography sampling systems consist of a high pressure sample valve that may also have the sample supplied to the valve from an automatic sampling device. Both gas and liquid chromatography have ovens that are usually temperature programmable, the temperature of the gas chromatographic oven range from 5°C to 350°C and the liquid chromatography oven from about 5°C to 120°C. Gas chromatography usually employs capillary columns whereas packed columns are the most common type of liquid chromatography column. The most common gas chromatography detectors are the flame ionization detector (FID) the

Nitrogen Phosphorous Detector (NPD) and the thermal conductivity detector or the hot wire detector (HWD). The three most common liquid chromatography detectors are the UV detector, the fluorescence detector and the refractive index detector (the RI detector). The data acquisition and processing system is very similar for both types of chromatography. They consist of a scaling amplifier and an A/D converter, the output of which is fed to a suitable computer and associated printer. Retention times, retention ratios, peak heights and peak widths are calculated and retention data and quantitative analyses are calculated and then printed out.

3. GAS CHROMATOGRAPHY

Gas Chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (an homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a solid and the mobile phase is a liquid. Hence the full name of the pro-

cedure is "gas–liquid chromatography", referring to the mobile and stationary phases, respectively. Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) does not have such a temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale (i.e. microscale).

Gas chromatography is also sometimes known as vapor-phase chromatography (VPC), or gas–liquid partition chromatography (GLPC). These alternative names, as well as their respective abbreviations, are frequently used in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors.

3.1 Gas Chromatograph

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature.

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and

on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.

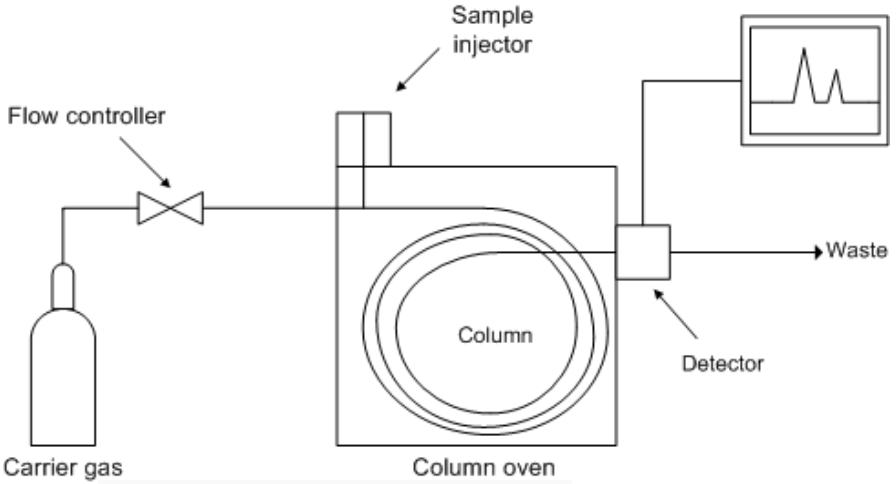


Fig.2 Diagram of a gas chromatograph.

3.2 Qualitative Analysis

Generally chromatographic data is presented as a graph of detector response (y-axis) against retention time (x-axis), which is called a chromatogram. This provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from the column at different times. Retention time can be used to identify analytes if the method conditions are constant. Also, the pattern of peaks will be constant for a sample under constant conditions and can identify complex mixtures of analytes. In most modern applications, however, the GC is connected to a mass spectrometer or similar detector that is capable of identifying the analytes represented by the peaks.

3.3 Quantitative Analysis

The area under a peak is proportional to the amount of analyte present in the chromatogram. By calculating the area of the peak using the mathematical function of integration, the concentration of an analyte in the original sample can be determined. Concentration can be calculated using a calibration curve created by finding the response for a series of concentrations of analyte, or by determining the relative response factor of an analyte. The relative response factor is the expected ratio of an analyte to an internal standard (or external standard) and is calculated by finding the response of a known amount of analyte and a constant amount of internal standard (a chemical added to the sample at a constant concentration, with a distinct retention time to the analyte).

In most modern gas chromatography-mass spectrometry (GC-MS) systems, computer software is used to draw and integrate peaks, and match MS spectra to library spectra.

3.4 Application

In general, substances that vaporize below ca. 300°C (and, therefore, are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they should not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.

Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process.

Professionals working with GC analyze the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water. GC is very accurate if used properly and can measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples.

In practical courses at colleges, students sometimes get acquainted to the GC by studying the contents of Lavender oil or measuring the ethylene that is secreted by Nicotiana benthamiana plants after artificially injuring their leaves. These GC analyses hydrocarbons (C2-C40+). In a typical experiment, a packed column is used to separate the light gases,

which are then detected with a Thermal Conductivity Detector (TCD). The hydrocarbons are separated using a capillary column and detected with an Flame Ionization Detector FID. A complication with light gas analyses that include H_2 is that He, which is the most common and most sensitive inert carrier (sensitivity is proportional to molecular mass) has an almost identical thermal conductivity to hydrogen (it is the difference in thermal conductivity between two separate filaments in a Wheatstone Bridge type arrangement that shows when a component has been eluted). For this reason, dual TCD instruments are used with a separate channel for hydrogen that uses nitrogen as a carrier are common. Argon is often used when analysing gas phase chemistry reactions such as F-T synthesis so that a single carrier gas can be used rather than 2 separate ones. The sensitivity is less but this is a tradeoff for simplicity in the gas supply.

4. LIQUID CHROMATOGRAPHY

Liquid chromatography (LC, HPLC) is a method of separation which employs a liquid mobile phase and either a solid (LSC) or a liquid (LLC) immobilized on a solid. A liquid may be immobilized on the solid by chemical reaction with the solid, by reaction to form an insoluble polymer deposited on the surface or it may be a liquid that is insoluble in the mobile phases to be used. The latter case is not used very much in current liquid chromatography systems but was important in the early days of LLC. Liquid chromatography is very versatile and is used to separate a wide variety of compounds ranging from small organic molecules to large polymers.

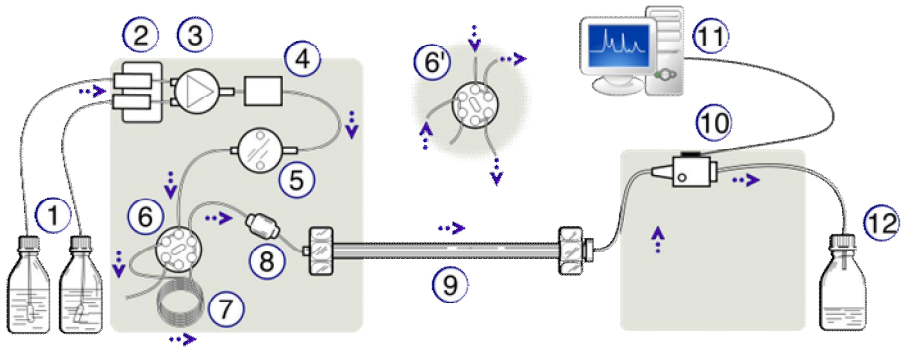
4.1 High Performance Liquid Chromatography

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture. Some common examples are the separation and quantitation of performance enhancement drugs (e.g. steroids) in urine samples, or of vitamin D levels in serum.

HPLC typically utilizes different types of stationary phases (i.e. sorbents) contained in columns, a pump that moves the mobile phase and sample components through the column, and a detector capable of provid-

ing characteristic retention times for the sample components and area counts reflecting the amount of each analyte passing through the detector. The detector may also provide additional information related to the analyte (i.e. UV/Vis spectroscopic data, if so equipped). Analyte retention time varies depending on the strength of its interactions with the stationary phase, the composition and flow rate of mobile phase used, and on the column dimensions. HPLC is a form of liquid chromatography that utilizes small size columns (typically 250.0 mm or shorter and 4.6 mm i.d. or smaller; packed with smaller particles), and higher mobile phase pressures compared to ordinary liquid chromatography.

With HPLC, a pump (rather than gravity) provides the higher pressure required to move the mobile phase and sample components through the densely packed column. The increased density arises from the use of smaller sorbent particles. Such particles are capable of providing better separation on columns of shorter length when compared to ordinary column chromatography.



(1) Solvent reservoirs, (2) Solvent degasser, (3) Gradient valve, (4) Mixing vessel for delivery of the mobile phase, (5) High-pressure pump, (6) Switching valve in "inject position", (6') Switching valve in "load position", (7) Sample injection loop, (8) Pre-column (guard column), (9) Analytical column, (10) Detector (i.e. IR, UV), (11) Data acquisition, (12) Waste or fraction collector.

Fig.3 Schematic representation of an HPLC unit.

4.2 Operation

The sample to be separated and analyzed is introduced, in a discrete small volume, into the stream of mobile phase percolating through the col-

umn. The components of the sample move through the column at different velocities function of specific physical or chemical interactions with the stationary phase. The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called the retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte. The use of smaller particle size packing materials requires the use of higher operational pressure (back pressure) and typically improves chromatographic resolution (i.e. the degree of separation between consecutive analytes emerging from the column). Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water free mobile phases (see Normal Phase HPLC below). The aqueous component of the mobile phase may contain buffers, acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components. The composition of the mobile phase may be kept constant (isocratic elution mode) or varied (gradient elution mode) during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very dissimilar in their affinity for the stationary phase.

In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes. Period of constant mobile phase composition may be part of any gradeint profile. For example, the mobile phase composition may be kept constant at 5% acetonitrile for 1-3 min, followed by a linear change up to 95% acetonitrile. The composition of the mobile phase depends on the intensity of interactions between analytes and stationary phase (e.g. hydrophobic interactions in reversed-phase HPLC). Depending on their affinity for the stationary and mobile phases analytes partition between the two during the separation process taking place in the column. This partitioning process is similar to that which occurs during a liquid-liquid extraction but is continuous, not step-wise. In this example, using a water/acetonitrile gradient, more hydrophobic components will elute (come off the column) late, once

the mobile phase gets more concentrated in acetonitrile (i.e. in a mobile phase of higher eluting strength).

The choice of mobile phase components, additives (such as salts or acids) and gradient conditions depend on the nature of the column and sample components. Often a series of trial runs are performed with the sample in order to find the HPLC method which gives the best separation.

4.3 Partition Chromatography

Partition chromatography was the first kind of chromatography that chemists developed. The partition coefficient principle has been applied in paper chromatography, thin layer chromatography, gas phase and liquid-liquid applications. The 1952 Nobel Prize in chemistry was earned by Archer John Porter Martin and Richard Laurence Millington Synge for their development of the technique, which was used for their separation of amino acids. Partition chromatography uses a retained solvent, on the surface or within the grains or fibers of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some coulombic and/or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent. Known as Hydrophilic Interaction Chromatography (HILIC) in HPLC, this method separates analytes based on polar differences. HILIC most often uses a bonded polar stationary phase and water miscible, high organic concentration, mobile phases. Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences. HILIC bonded phases have the advantage of separating acidic, basic and neutral solutes in a single chromatogram.

The polar analytes diffuse into a stationary water layer associated with the polar stationary phase and are thus retained. Retention strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends on the functional groups in the analyte molecule which promote partitioning but can also include coulombic (electrostatic) interaction and hydrogen donor capability.

Use of more polar solvents in the mobile phase will decrease the retention time of the analytes, whereas more hydrophobic solvents tend to increase retention times.

4.4 Normal-Phase Chromatography

It was one of the first kinds of HPLC that chemists developed. Also known as normal-phase HPLC (NP-HPLC), or adsorption chromatography, this method separates analytes based on their affinity for a polar stationary surface such as silica, hence it is based on analyte ability to engage in polar interactions (such as hydrogen-bonding or dipole-dipole type of interactions) with the sorbent surface. NP-HPLC uses a non-polar, non-aqueous mobile phase, and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity. The interaction strength depends not only on the functional groups present in the structure of the analyte molecule, but also on steric factors. The affect of steric hindrance on interaction strength allows this method to resolve (separate) structural isomers.

The use of more polar solvents in the mobile phase will decrease the retention time of analytes, whereas more hydrophobic solvents tend to induce slower elution (increased retention times). Very polar solvents such as traces of water in the mobile phase tend to adsorb to the solid surface of the stationary phase forming a stationary bound (water) layer which is considered to play an active role in retention. This behavior is somewhat peculiar to normal phase chromatograhy because it is governed almost exclusively by an adsorptive mechanism (i.e. analytes interact with a solid surface rather than with the solvated layer of a ligand attached to the sorbent surface; see also reversed-phase HPLC below). Adsorption chromatography is still widely used for structural isomer separations in both column and thin-layer chromatography formats on activated (dried) silica or alumina supports.

Partition and NP-HPLC fell out of favor in the 1970s with the development of reversed-phase HPLC because of poor reproducibility of retention times due to the presence of a water or protic organic solvent layer on the surface of the silica or alumina chromatographic media. This layer changes with any changes in the composition of the mobile phase (e.g. moisture level) causing drifting retention times.

Recently, partition chromatography has become popular again with the development of HILIC bonded phases which demonstrate improved reproducibility, and due to a better understanding of the range of usefulness of the technique.

4.5 Displacement Chromatography

The basic principle of displacement chromatography is: A molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities. There are distinct differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than "peaks". Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

4.6 Reversed-Phase Chromatography (RPC)

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RP-HPLC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release.

RP-HPLC operates on the principle of hydrophobic interactions, which originate from the high symmetry in the dipolar water structure and play the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C_{18}-chain versus the complex of both. The energy released in this process is proportional to the surface tension of the eluent (water: 7.3×10^{-6} J/cm², methanol: 2.2×10^{-6} J/cm²) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding a less polar solvent (methanol, acetonitrile) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis.

Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) is retained longer because it is non-interacting with the water structure. On the other hand, analytes with higher polar surface area (conferred by the presence of polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ in their structure) are less retained as they are better integrated into water. Such interactions are subject to steric effects in that very large molecules may have only restricted access to the pores of the stationary phase, where the interactions with surface ligands (alkyl chains) take place. Such surface hindrance typically results in less retention.

Retention time increases with hydrophobic (non-polar) surface area. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased. Similarly organic compounds with single C-C-bonds elute later than those with a C=C or C-C-triple bond, as the double or triple bond is shorter than a single C-C-bond.

Aside from mobile phase surface tension (organizational strength in eluent structure), other mobile phase modifiers can affect analyte retention. For example, the addition of inorganic salts causes a moderate linear increase in the surface tension of aqueous solutions (ca. 1.5×10^{-7} J/cm² per Mol for NaCl, 2.5×10^{-7} J/cm² per Mol for $(NH_4)_2SO_4$), and because

the entropy of the analyte-solvent interface is controlled by surface tension, the addition of salts tend to increase the retention time. This technique is used for mild separation and recovery of proteins and protection of their biological activity in protein analysis (hydrophobic interaction chromatography, HIC).

Another important factor is the mobile phase pH since it can change the hydrophobic character of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. Buffers serve multiple purposes: control of pH, neutralize the charge on the silica surface of the stationary phase and act as ion pairing agents to neutralize analyte charge. Ammonium formate is commonly added in mass spectrometry to improve detection of certain analytes by the formation of analyte-ammonium adducts. A volatile organic acid such as acetic acid, or most commonly formic acid, is often added to the mobile phase if mass spectrometry is used to analyze the column effluent. Trifluoroacetic acid is used infrequently in mass spectrometry applications due to its persistence in the detector and solvent delivery system, but can be effective in improving retention of analytes such as carboxylic acids in applications utilizing other detectors, as it is a fairly strong organic acid. The effects of acids and buffers vary by application but generally improve chromatographic resolution.

Reversed phase columns are quite difficult to damage compared with normal silica columns; however, many reversed phase columns consist of alkyl derivatized silica particles and should never be used with aqueous bases as these will destroy the underlying silica particle. They can be used with aqueous acid, but the column should not be exposed to the acid for too long, as it can corrode the metal parts of the HPLC equipment. RP-HPLC columns should be flushed with clean solvent after use to remove residual acids or buffers, and stored in an appropriate composition of solvent. The metal content of HPLC columns must be kept low if the best possible ability to separate substances is to be retained. A good test for the metal content of a column is to inject a sample which is a mixture of 2,2'- and 4,4'- bipyridine. Because the 2,2'-bipy can chelate the metal, the shape of the peak for the 2,2'-bipy will be distorted (tailed) when metal ions are present on the surface of the silica.

4.7 Size-Exclusion Chromatography

Size-exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography, separates particles on

the basis of size. It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins. SEC is used primarily for the analysis of large molecules such as proteins or polymers. SEC works by trapping these smaller molecules in the pores of a particle. The larger molecules simply pass by the pores as they are too large to enter the pores. Larger molecules therefore flow through the column quicker than smaller molecules, that is, the smaller the molecule, the longer the retention time.

This technique is widely used for the molecular weight determination of polysaccharides. SEC is the official technique (suggested by European pharmacopeia) for the molecular weight comparison of different commercially available low-molecular weight heparins.

4.8 Ion-Exchange Chromatography

In ion-exchange chromatography (IC), retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. Types of ion exchangers include:

- Polystyrene resins – These allow cross linkage which increases the stability of the chain. Higher cross linkage reduces swerving, which increases the equilibration time and ultimately improves selectivity.
- Cellulose and dextran ion exchangers (gels) – These possess larger pore sizes and low charge densities making them suitable for protein separation.
- Controlled-pore glass or porous silica.

In general, ion exchangers favor the binding of ions of higher charge and smaller radius.

An increase in counter ion (with respect to the functional groups in resins) concentration reduces the retention time. A decrease in pH reduces the retention time in cation exchange while an increase in pH reduces the retention time in anion exchange. By lowering the pH of the solvent in a cation exchange column, for instance, more hydrogen ions are available to compete for positions on the anionic stationary phase, thereby eluting weakly bound cations.

This form of chromatography is widely used in the following applications: water purification, preconcentration of trace components, ligand-exchange chromatography, ion-exchange chromatography of proteins, high-

pH anion-exchange chromatography of carbohydrates and oligosaccharides, and others.

4.9 Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include: analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, assaying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents.

Depending on the nature of the stationary phase can be implemented the following types of thin-layer chromatography of polymers:

- adsorptional thin-layer chromatography is based on the separation of macromolecules according to their adsorption activity, which increases with increasing molecular weight or the proportion of adsorption-active polar groups;

- sedimentary thin-layer chromatography is based on using as eluent the mixtures of solvents with a large number of adsorption-active polymer precipitant. Separation occurs due to changes in eluent dissolving properties along the length of the chromatographic plate. This change may be due to the fact that on the plate is fed eluent of changing composition or the fact that the composition of the eluent is changed directly on the plate as a result of evaporation and reducing the phase mass ratio of the eluent and the adsorbent;

- extractive thin-layer chromatography is based on the selective dissolution of polymer in the starting spot on the principle "all or nothing". It is used one-component solvent, which allows dividing the polymer fractions in the starting spot;

- thin-layer electrophoresis is electrophoretic migrations (separations) through athin layer of inert material, such as cellulose, supported on a glass or plastic plate;

- isoelectric focusing - electrophoresis on a layer with a gradient of pH;

- thin-layer gel permeation chromatography is based on molecular-sieve effect, which can occur under two conditions: the suppression of the adsorption activity of the adsorbent and then filling it with a solvent. Pore size is determined by the degree of macromolecules crosslinking in the gel.

Technique

The process is similar to paper chromatography with the advantage of faster runs, better separations, and the choice between different stationary phases. Because of its simplicity and speed TLC is often used for monitoring chemical reactions and for the qualitative analysis of reaction products.

To run a thin layer chromatography, the following procedure is carried out:

- A small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber.
- A small amount of an appropriate solvent (elutant) is poured in to a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1 centimeter. A strip of filter paper is put into the chamber, so that its bottom touches the solvent, and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapors ascend the filter paper and saturate the air in the chamber. (Failure to saturate the chamber will result in poor separation and non-reproducible results).
- The TLC plate (see Fig.4) is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the elutant in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). When the solvent front reaches no higher than the top of the filter pa-

per in the chamber, the plate should be removed (continuation of the elution will give a misleading result) and dried.

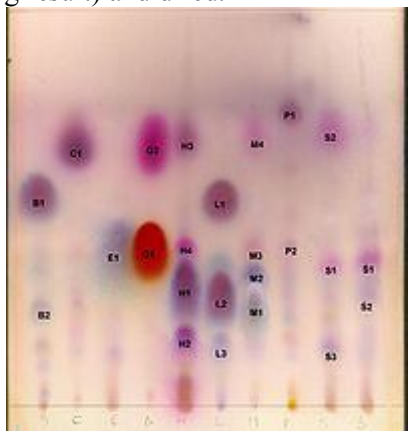


Fig.4 TLC plate.

As the chemicals being separated may be colorless, several methods exist to visualize the spots:

- Often a small amount of a fluorescent compound, usually manganese-activated zinc silicate, is added to the adsorbent that allows the visualization of spots under a blacklight (UV_{254}). The adsorbent layer will thus fluoresce to light green by itself, but spots of analyte quench this fluorescence.
- Iodine vapors are a general unspecific color reagent.
- Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate.
- Potassium permanganate – oxidation.
- In the case of lipids, the chromatogram may be transferred to a PVDF membrane and then subjected to further analysis, for example mass spectrometry, a technique known as Far-Eastern blotting.

Once visible, the R_f value, or retardation factor, of each spot can be determined by dividing the distance the product traveled from the initial spotting site by the distance the solvent front traveled from the initial spotting site. These values depend on the solvent used, and the type of TLC plate, and are not physical constants. Eluent on the thin layer is put on top of the plate.

4.10 Gel Permeation Chromatography (Size Exclusion)

Gel permeation chromatography (GPC) is a type of size exclusion chromatography (SEC) that separates analytes on the basis of size. The technique is often used for the analysis of polymers. As a technique, SEC was first developed in 1955 by Lathe and Ruthven. The term gel permeation chromatography can be traced back to J.C. Moore of the Dow Chemical Company who investigated the technique in 1964. It is often necessary to separate polymers, both to analyze them as well as to purify the desired product.

When characterizing polymers, it is important to consider the polydispersity index (PDI) as well the molecular weight. Polymers can be characterized by a variety of definitions for molecular weight including the number average molecular weight (M_n), the average molecular weight (M_w) (see molar mass distribution), the size average molecular weight (M_z), or the viscosity molecular weight (M_v). GPC allows for the determination of PDI as well as M_v and based on other data, the M_n, M_w, and M_z can be determined.

GPC separates based on the size or hydrodynamic volume (radius of gyration) of the analytes. This differs from other separation techniques which depend upon chemical or physical interactions to separate analytes. Separation occurs via the use of porous beads packed in a column (see Fig.5). The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. Conversely, larger analytes spend little if any time in the pores and are eluted quickly. All columns have a range of molecular weights that can be separated.

If an analyte is either too large or too small it will be either not retained or completely retained respectively. Analytes that are not retained are eluted with the free volume outside of the particles (V_o), while analytes that are completely retained are eluted with volume of solvent held in the pores (V_i). The total volume can be considered by the following equation, where V_g is the volume of the polymer gel and V_t is the total volume:

$$Vt = Vg + Vi + Vo$$

As can be inferred, there is a limited range of molecular weights that can be separated by each column and therefore the size of the pores for the packing should be chosen according to the range of molecular weight of analytes to be separated. For polymer separations the pore sizes should be on the order of the polymers being analyzed. If a sample has a broad mole-

cular weight range it may be necessary to use several GPC columns in tandem with one another to fully resolve the sample.

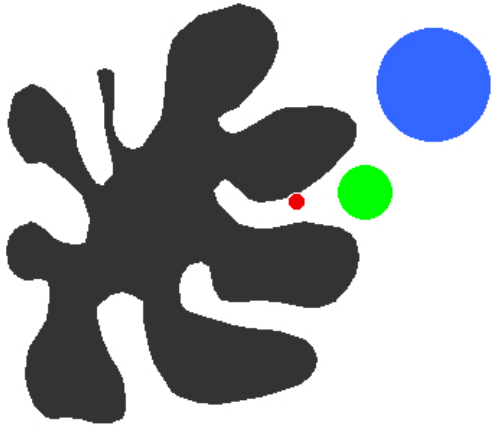


Fig.5 Principle of GPC separation.

GPC is often used to determine the relative molecular weight of polymer samples as well as the distribution of molecular weights. What GPC truly measures is the molecular volume and shape function as defined by the intrinsic viscosity. If comparable standards are used, this relative data can be used to determine molecular weights within ± 5% accuracy. Polystyrene standards with PDI of less than 1.2 are typically used to calibrate the GPC. Unfortunately, polystyrene tends to be a very linear polymer and therefore as a standard it is only useful to compare it to other polymers that are known to be linear and of relatively the same size.

Material and Methods

The most widely used sorbent gels are hydrophobic materials such as polystyrene or crosslinked divinylbenzene. In these gels is practically completely absent the effects of analyzed samples adsorption. Recently are widespread macroporous glasses.

Solvents for gel permeation chromatography are tetrahydrofuran, chloroform, toluene, cyclohexane and their mixtures.

The detectors used in gel permeation chromatography are UV detector, refractometer, flow viscometer. The combination of two detectors is useful for the analysis of new polymers or macromolecular complex structure, molecular and compositional heterogeneity of copolymers. Typically, both detectors are mounted in a chromatograph, and analyzed the polymer solution successively transferred from one detector to another,

which allows immediately build an integral or differential distribution curve by the composition of the sample.

The columns are tubes made of stainless steel, ranging in length from 20 to 200 mm and a diameter of 2 mm to 10 mm. Each filled column should be calibrated by passing through it polymer samples with known molecular weights.

Advantages and Disadvantages

As a separation technique GPC has many advantages. First of all, it has a well-defined separation time due to the fact that there is a final elution volume for all unretained analytes. Additionally, GPC can provide narrow bands, although this aspect of GPC is more difficult for polymer samples that have broad ranges of molecular weights present. Finally, since the analytes do not interact chemically or physically with the column, there is a lower chance for analyte loss to occur. For investigating the properties of polymer samples in particular, GPC can be very advantageous. GPC provides a more convenient method of determining the molecular weights of polymers. In fact most samples can be thoroughly analyzed in an hour or less. Other methods used in the past were fractional extraction and fractional precipitation. As these processes were quite labor intensive molecular weights and mass distributions typically were not analyzed. Therefore, GPC has allowed for the quick and relatively easy estimation of molecular weights and distribution for polymer samples.

There are disadvantages to GPC, however. First, there are a limited number of peaks that can be resolved within the short time scale of the GPC run. Also, as a technique GPC requires around at least a 10% difference in molecular weight for a reasonable resolution of peaks to occur. In regards to polymers, the molecular masses of most of the chains will be too close for the GPC separation to show anything more than broad peaks. Another disadvantage of GPC for polymers is that filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors. Although useful for protecting the instrument, the pre-filtration of the sample has the possibility of removing higher molecular weight sample before it can be loaded on the column.

5. MASS-SPECTROMETRY

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios. In a typical MS procedure:

1. A sample is loaded onto the MS instrument and undergoes vaporization.
2. The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions).
3. The ions are separated according to their mass-to-charge ratio in an analyzer by electromagnetic fields.
4. The ions are detected, usually by a quantitative method.
5. The ion signal is processed into mass spectra.

MS instruments consist of three modules:

- An ion source, which can convert gas phase sample molecules into ions (or, in the case of electrospray ionization, move ions that exist in solution into the gas phase).
- A mass analyzer, which sorts the ions by their masses by applying electromagnetic fields.
- A detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present.

The technique has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

5.1 Simplified Example

The following example describes the operation of a spectrometer mass analyzer, which is of the sector type. (Other analyzer types are treated below.) Consider a sample of sodium chloride (table salt). In the ion source, the sample is vaporized (turned into gas) and ionized (transformed into

electrically charged particles) into sodium (Na^+) and chloride (Cl^-) ions. Sodium atoms and ions are monoisotopic, with a mass of about 23 amu. Chloride atoms and ions come in two isotopes with masses of approximately 35 amu (at a natural abundance of about 75 percent) and approximately 37 amu (at a natural abundance of about 25 percent). The analyzer part of the spectrometer contains electric and magnetic fields, which exert forces on ions traveling through these fields. The speed of a charged particle may be increased or decreased while passing through the electric field, and its direction may be altered by the magnetic field. The magnitude of the deflection of the moving ion's trajectory depends on its mass-to-charge ratio. Lighter ions get deflected by the magnetic force more than heavier ions (based on Newton's second law of motion, F = ma). The streams of sorted ions pass from the analyzer to the detector, which records the relative abundance of each ion type. This information is used to determine the chemical element composition of the original sample (i.e. that both sodium and chlorine are present in the sample) and the isotopic composition of its constituents (the ratio of ^{35}Cl to ^{37}Cl).

5.2 Creating Ions

The ion source is the part of the mass spectrometer that ionizes the material under analysis (the analyte). The ions are then transported by magnetic or electric fields to the mass analyzer.

Techniques for ionization have been key to determining what types of samples can be analyzed by mass spectrometry. Electron ionization and chemical ionization are used for gases and vapors. In chemical ionization sources, the analyte is ionized by chemical ion-molecule reactions during collisions in the source. Two techniques often used with liquid and solid biological samples include electrospray ionization (invented by John Fenn) and matrix-assisted laser desorption/ionization (MALDI, initially developed as a similar technique "Soft Laser Desorption (SLD)" by K. Tanaka for which a Nobel Prize was awarded and as MALDI by M. Karas and F. Hillenkamp).

Inductively coupled plasma (ICP) sources are used primarily for cation analysis of a wide array of sample types. In this type of Ion Source Technology, a 'flame' of plasma that is electrically neutral overall, but that has had a substantial fraction of its atoms ionized by high temperature, is used to atomize introduced sample molecules and to further strip the outer electrons from those atoms. The plasma is usually generated from argon

gas, since the first ionization energy of argon atoms is higher than the first of any other elements except He, O, F and Ne, but lower than the second ionization energy of all except the most electropositive metals. The heating is achieved by a radio-frequency current passed through a coil surrounding the plasma.

Others include glow discharge, field desorption (FD), fast atom bombardment (FAB), thermospray, desorption/ionization on silicon (DIOS), Direct Analysis in Real Time (DART), atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS), spark ionization and thermal ionization (TIMS). Ion attachment ionization is an ionization technique that allows for fragmentation free analysis.

5.3 Types of Mass Analyzers

Mass analyzers separate the ions according to their mass-to-charge ratio. The following two laws govern the dynamics of charged particles in electric and magnetic fields in vacuum:

$F = Q(E + v \times B)$ (Lorentz force law);

$F = ma$ (Newton's second law of motion in non-relativistic case, i.e. valid only at ion velocity much lower than the speed of light).

Here F is the force applied to the ion, m is the mass of the ion, a is the acceleration, Q is the ion charge, E is the electric field, and $v \times B$ is the vector cross product of the ion velocity and the magnetic field.

Equating the above expressions for the force applied to the ion yields:

$(m/Q)a = E + v \times B$

This differential equation is the classic equation of motion for charged particles. Together with the particle's initial conditions, it completely determines the particle's motion in space and time in terms of m/Q. Thus mass spectrometers could be thought of as "mass-to-charge spectrometers". When presenting data, it is common to use the (officially) dimensionless m/z, where z is the number of elementary charges (e) on the ion (z=Q/e). This quantity, although it is informally called the mass-to-charge ratio, more accurately speaking represents the ratio of the mass number and the charge number, z.

There are many types of mass analyzers, using both static or dynamic fields, and magnetic or electric fields, but all operate according to the above differential equation. Each analyzer type has its strengths and weaknesses. Many mass spectrometers use two or more mass analyzers

for tandem mass spectrometry (MS/MS). In addition to the more common mass analyzers listed below, there are others designed for special situations.

There are several important analyser characteristics. The mass resolving power is the measure of the ability to distinguish two peaks of slightly different m/z. The mass accuracy is the ratio of the m/z measurement error to the true m/z. Mass accuracy is usually measured in ppm or milli mass units. The mass range is the range of m/z amenable to analysis by a given analyzer. The linear dynamic range is the range over which ion signal is linear with analyte concentration. Speed refers to the time frame of the experiment and ultimately is used to determine the number of spectra per unit time that can be generated.

Quadrupole mass analyzers use oscillating electrical fields to selectively stabilize or destabilize the paths of ions passing through a radio frequency (RF) quadrupole field created between 4 parallel rods. Only the ions in a certain range of mass/charge ratio are passed through the system at any time, but changes to the potentials on the rods allow a wide range of m/z values to be swept rapidly, either continuously or in a succession of discrete hops. A quadrupole mass analyzer acts as a mass-selective filter and is closely related to the quadrupole ion trap, particularly the linear quadrupole ion trap except that it is designed to pass the untrapped ions rather than collect the trapped ones, and is for that reason referred to as a transmission quadrupole. A common variation of the transmission quadrupole is the triple quadrupole mass spectrometer. The "triple quad" has three consecutive quadrupole stages, the first acting as a mass filter to transmit a particular incoming ion to the second quadrupole, a collision chamber, wherein that ion can be broken into fragments. The third quadrupole also acts as a mass filter, to transmit a particular fragment ion to the detector. If a quadrupole is made to rapidly and repetitively cycle through a range of mass filter settings, full spectra can be reported. Likewise, a triple quad can be made to perform various scan types characteristic of tandem mass spectrometry.

5.4 Application

Isotope Ratio MS: Isotope Dating and Tracking

Mass spectrometry is also used to determine the isotopic composition of elements within a sample. Differences in mass among isotopes of an element are very small, and the less abundant isotopes of an element are typically very rare, so a very sensitive instrument is required. These instruments, sometimes referred to as isotope ratio mass spec-

trometers (IR-MS), usually use a single magnet to bend a beam of ionized particles towards a series of Faraday cups which convert particle impacts to electric current. A fast on-line analysis of deuterium content of water can be done using Flowing afterglow mass spectrometry, FA-MS. Probably the most sensitive and accurate mass spectrometer for this purpose is the accelerator mass spectrometer (AMS). Isotope ratios are important markers of a variety of processes. Some isotope ratios are used to determine the age of materials for example as in carbon dating. Labeling with stable isotopes is also used for protein quantification.

Trace Gas Analysis

Several techniques use ions created in a dedicated ion source injected into a flow tube or a drift tube: selected ion flow tube (SIFT-MS), and proton transfer reaction (PTR-MS), are variants of chemical ionization dedicated for trace gas analysis of air, breath or liquid headspace using well defined reaction time allowing calculations of analyte concentrations from the known reaction kinetics without the need for internal standard or calibration.

Atom Probe

An atom probe is an instrument that combines time-of-flight mass spectrometry and field ion microscopy (FIM) to map the location of individual atoms.

Polymer Characterization

The characterization of polymers presents particular problems for analytical techniques. In addition to their high molecular weights, most polymers are polydisperse and cover an extremely wide range of molecular weights, with hundreds or thousands of different molecular weight species. Furthermore, additional distributions in composition, end-groups and topology, are superimposed on the molecular weight distribution.

Mass spectrometry can be used to accurately measure molecular masses, however, the difficulty in applying this technique to polymers has been volatizing high molecular mass polymeric ions without fragmentation. Although analysis of the spectra of fragmented polymers provides considerable information about the polymer, it does not allow the chain length, and chain length distribution, to be measured.

Recent advances in soft ionization mass spectrometry, in which only intact molecular ions are observed with minimal fragmentation, affords both the chain length distribution and the chemical composition at every chain length, which may be deduced from the mass. The complete distribution of chains (length, monomers, end-groups) can ideally be identified and quantified in a single experiment which usually takes under ten minutes to perform, and uses only sub-microgram quantities of materials. The mass spectrometry data are also predictable as both the exact mass (monoisotopic) and isotope patterns can be computed prior to analysis if the composition is known. In addition, on-line databases, such as the Science and Technology Network, can facilitate identification and retrieve patent information by searching the formula weight or molecular formula furnished by mass spectrometry. This is particularly useful for industrial chemists.

Ideally, mass spectrometry provides a quantitative measure of the molecular weight distribution of the polymer sample as well as composition and end-group distributions. The accuracy of these distributions, and the molecular weight averages derived from them, depends upon uniform measurement efficiency across the relevant molecular weight range. The measurement efficiency for a given polymeric species in a distribution depends on a number of factors, including the efficiency of dissolving the sample in the matrix, sample vaporization, ionization, ion transmission and finally detection.

6. SPECTROSCOPY

Spectroscopy is the study of the interaction between matter and radiated energy. Historically, spectroscopy originated through the study of visible light dispersed according to its wavelength, e.g., by a prism. Later the concept was expanded greatly to comprise any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopic data is often represented by a spectrum, a plot of the response of interest as a function of wavelength or frequency.

Spectrometry and spectrography are terms used to refer to the measurement of radiation intensity as a function of wavelength and are often used to describe experimental spectroscopic methods. Spectral measurement devices are referred to as spectrometers, spectrophotometers, spectrographs or spectral analyzers.

Table 1. Electromagnetic Spectrum

Region of the Spectrum	Applications for Scientific Purposes	ν, Hz	λ, nm
Cosmic rays	-	10^{22}	$3 \cdot 10^{-3}$
Gamma radiation	Nuclear transitions	10^{19}	0,3
Soft X-rays	Transitions of inner electrons of atoms	10^{17}	30
Vacuum ultraviolet region	Transitions of valence electrons (electronic spectra)	$1.5 \cdot 10^{15}$	200
Quartz UV region		$7.5 \cdot 10^{14}$	400
Visible area		$3.8 \cdot 10^{14}$	800
Near-infrared	Vibrational IR and Raman bands over tones	$1.2 \cdot 10^{14}$	2500
Average IR region	The main area of vibrational transitions	$2.5 \cdot 10^{13}$	12500
Far-infrared	Skeletal vibrations of molecules, hindered rotation cycles, the deformation of solids	10^{12}	300000
Microwave (microwave currents)	Rotation of the molecules, inhibited internal rotation relative to chemical bonds	10^{9}	300 мм
Short radio waves	Reorientation of the spin (NMR, EPR)	$1.5 \cdot 10^{6}$	200 m
Broadcasting	Radio, TV	$5.5 \cdot 10^{5}$	550 m
Long radio waves	Induction heating, long-wave communication	$3 \cdot 10^{3}$	10 km
Electrical industry	Power energy, lighting	0.3	10^{6} km

Daily observations of color can be related to spectroscopy. Neon lighting is a direct application of atomic spectroscopy. Neon and other noble gases have characteristic emission colors, and neon lamps use electricity to excite these emissions. Inks, dyes and paints include chemical

compounds selected for their spectral characteristics in order to generate specific colors and hues. A commonly encountered molecular spectrum is that of nitrogen dioxide. Gaseous nitrogen dioxide has a characteristic red absorption feature, and this gives air polluted with nitrogen dioxide a reddish brown color. Rayleigh scattering is a spectroscopic scattering phenomenon that accounts for the color of the sky.

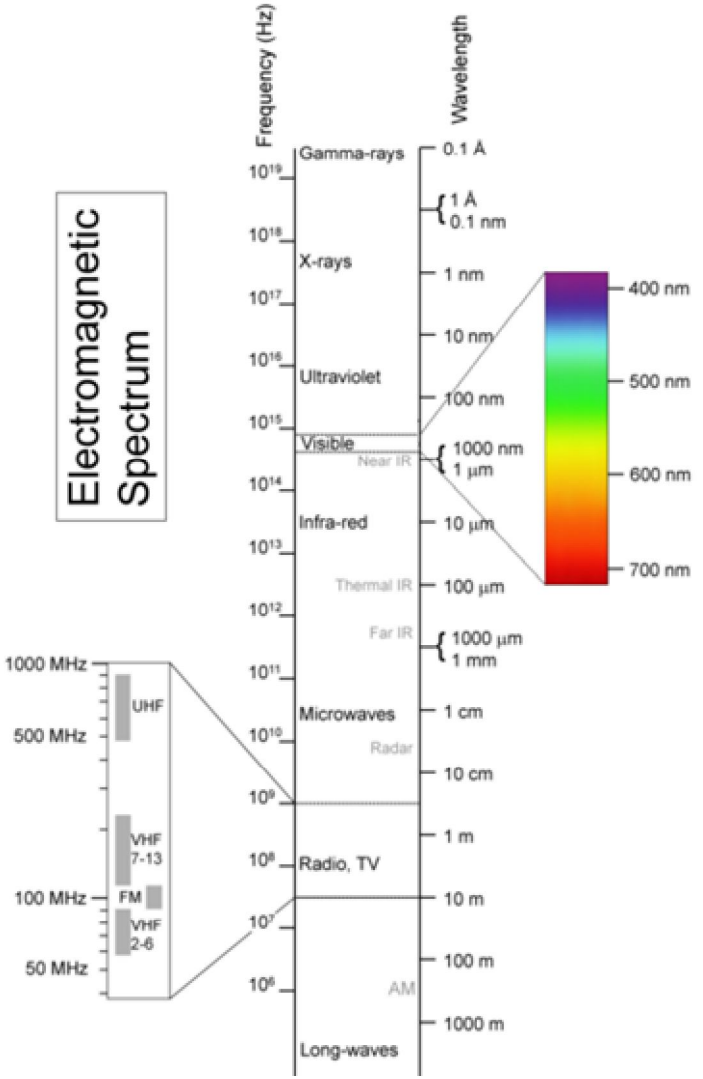


Fig. 6 Electromagnetic spectrum.

Spectroscopic studies were central to the development of quantum mechanics and included Max Planck's explanation of blackbody radiation, Albert Einstein's explanation of the photoelectric effect and Niels Bohr's explanation of atomic structure and spectra. Spectroscopy is used in physical and analytical chemistry because atoms and molecules have unique spectra. These spectra can be interpreted to derive information about the atoms and molecules, and they can also be used to detect, identify and quantify chemicals. Spectroscopy is also used in astronomy and remote sensing. Most research telescopes have spectrographs. The measured spectra are used to determine the chemical composition and physical properties of astronomical objects (such as their temperature and velocity).

6.1 Theory

One of the central concepts in spectroscopy is a resonance and its corresponding resonant frequency. Resonances were first characterized in mechanical systems such as pendulums. Mechanical systems that vibrate or oscillate will experience large amplitude oscillations when they are driven at their resonant frequency. A plot of amplitude vs. excitation frequency will have a peak centered at the resonance frequency. This plot is one type of spectrum, with the peak often referred to as a spectral line, and most spectral lines have a similar appearance.

In quantum mechanical systems, the analogous resonance is a coupling of two quantum mechanical stationary states of one system, such as an atom, via an oscillatory source of energy such as aphoton. The coupling of the two states is strongest when the energy of the source matches the energy difference between the two states. The energy (E) of a photon is related to its frequency (v) by $E+hv$ where h is Planck's constant, and so a spectrum of the system response vs. photon frequency will peak at the resonant frequency or energy. Particles such as electrons and neutrons have a comparable relationship, the de Broglie relations, between their kinetic energy and their wavelength and frequency and therefore can also excite resonant interactions.

Spectra of atoms and molecules often consist of a series of spectral lines, each one representing a resonance between two different quantum states. The explanation of these series, and the spectral patterns associated with them, were one of the experimental enigmas that drove the development and acceptance of quantum mechanics. The hydrogen spectral series in particular was first successfully explained by the Rutherford-Bohr quantum model of the hydrogen atom. In some cases spectral lines are well separated and distinguishable, but spectral lines can also overlap and appear to be a single transition if the density of energy states is high enough.

Spectroscopy is a sufficiently broad field that many sub-disciplines exist, each with numerous implementations of specific spectroscopic techniques. The various implementations and techniques can be classified in several ways.

6.2 Type of Radiative Energy

Types of spectroscopy are distinguished by the type of radiative energy involved in the interaction. In many applications, the spectrum is determined by measuring changes in the intensity or frequency of this energy. The types of radiative energy studied include:

- Electromagnetic radiation was the first source of energy used for spectroscopic studies. Techniques that employ electromagnetic radiation are typically classified by the wavelength region of the spectrum and include microwave, terahertz, infrared, near infrared, visible and ultraviolet, x-ray and gamma spectroscopy.
- Particles, due to their de Broglie wavelength, can also be a source of radiative energy and both electrons and neutrons are commonly used. For a particle, its kinetic energy determines its wavelength.
- Acoustic spectroscopy involves radiated pressure waves.
- Mechanical methods can be employed to impart radiating energy, similar to acoustic waves, to solid materials.

6.3 Nature of Interaction

Types of spectroscopy can also be distinguished by the nature of the interaction between the energy and the material. These interactions include:

- Absorption occurs when energy from the radiative source is absorbed by the material. Absorption is often determined by measuring the fraction of energy transmitted through the material; absorption will decrease the transmitted portion.
- Emission indicates that radiative energy is released by the material. A material's blackbody spectrum is a spontaneous emission spectrum determined by its temperature. Emission can also be induced by other sources of energy such as a flames or sparks or electromagnetic radiation in the case of fluorescence.

• Elastic scattering and reflection spectroscopy determine how incident radiation is reflected or scattered by a material. Crystallography employs the scattering of high energy radiation, such as x-rays and electrons, to examine the arrangement of atoms in proteins and solid crystals.

• Impedance spectroscopy studies the ability of a medium to impede or slow the transmittance of energy. For optical applications, this is characterized by the index of refraction.

• In elastic scattering phenomena involve an exchange of energy between the radiation and the matter that shifts the wavelength of the scattered radiation. These include Raman and Compton scattering.

• Coherent or resonance spectroscopy are techniques where the radiative energy couples two quantum states of the material in a coherent interaction that is sustained by the radiating field. The coherence can be disrupted by other interactions, such as particle collisions and energy transfer, and so often require high intensity radiation to be sustained. Nuclear magnetic resonance (NMR) spectroscopy is a widely used resonance method and ultrafast laser methods are also now possible in the infrared and visible spectral regions.

6.4 Type of Material

Spectroscopic studies are designed so that the radiant energy interacts with specific types of matter.

Atoms

Atomic spectroscopy was the first application of spectroscopy developed. Atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES) involve visible and ultraviolet light. These absorptions and emissions, often referred to as atomic spectral lines, are due to electronic transitions of an outer shell electron to an excited state. Atoms also have distinct x-ray spectra that are attributable to the excitation of inner shell electrons to excited states.

Atoms of different elements have distinct spectra and therefore atomic spectroscopy allows for the identification and quantitation of a sample's elemental composition. Robert Bunsen, developer of the Bunsen burner, and Gustav Kirchhoff discovered new elements by observing their emission spectra. Atomic absorption lines are observed in the solar spectrum and referred to as Fraunhofer lines after their discoverer. A comprehensive explanation of the hydrogen spectrum was an early success of quantum mechanics and explaining the Lamb shift observed in the hydrogen spectrum led to the development of quantum electrodynamics.

Modern implementations of atomic spectroscopy for studying visible and ultraviolet transitions include flame emission spectroscopy, inductively coupled plasma atomic emission spectroscopy, glow discharge spectroscopy, microwave induced plasma spectroscopy, and spark or arc emission spectroscopy. Techniques for studying x-ray spectra include X-ray spectroscopy and X-ray fluorescence (XRF).

Molecules

The combination of atoms into molecules leads to the creation of unique types of energetic states and therefore unique spectra of the transitions between these states. Molecular spectra can be obtained due to electron spin states (electron paramagnetic resonance), molecular rotations, molecular vibration and electronic states. Rotations are collective motions of the atomic nuclei and typically lead to spectra in the microwave and millimeter-wave spectral regions; rotational spectroscopy and microwave spectroscopy are synonymous. Vibrations are relative motions of the atomic nuclei and are studied by both infrared and Raman spectroscopy. Electronic excitations are studied using visible and ultraviolet spectroscopy as well as fluorescence spectroscopy.

Studies in molecular spectroscopy led to the development of the first maser and contributed to the subsequent development of the laser.

Crystals and Extended Materials

The combination of atoms or molecules into crystals or other extended forms leads to the creation of additional energetic states. These states are numerous and therefore have a high density of states. This high density often makes the spectra weaker and less distinct, i.e., broader. For instance, blackbody radiation is due to the thermal motions of atoms and molecules within a material. Acoustic and mechanical responses are due to collective motions as well.

Pure crystals, though, can have distinct spectral transitions and the crystal arrangement also has an effect on the observed molecular spectra. The regular lattice structure of crystals also scatters x-rays, electrons or neutrons allowing for crystallographic studies.

Nuclei

Nuclei also have distinct energy states that are widely separated and lead to gamma ray spectra. Distinct nuclear spin states can have their energy separated by a magnetic field, and this allows for NMR spectroscopy.

7. X-RAY PHOTOELECTRON SPECTROSCOPY

X-Ray Photoelectron Spectroscopy (XPS) is a quantitative spectroscopic technique that measures the elemental composition, empirical formula, chemical state and electronic state of the elements that exist within a material. XPS spectra are obtained by irradiating a material with a beam of X-rays while simultaneously measuring the kinetic energy and number of electrons that escape from the top 1 to 10 nm of the material being analyzed. XPS requires ultra-high vacuum (UHV) conditions.

XPS is a surface chemical analysis technique that can be used to analyze the surface chemistry of a material in its "as received" state, or after some treatment, for example: fracturing, cutting or scraping in air or UHV to expose the bulk chemistry, ion beam etchingto clean off some of the surface contamination, exposure to heat to study the changes due to heating, exposure to reactive gases or solutions, exposure to ion beam implant, exposure to ultraviolet light.

- XPS is also known as ESCA, an abbreviation for Electron Spectroscopy for Chemical Analysis.
- XPS detects all elements with an atomic number (Z) of 3 (lithium) and above. It cannot detect hydrogen (Z = 1) or helium (Z = 2) because the diameter of these orbitals is so small, reducing the catch probability to almost zero.
- Detection limits for most of the elements are in the parts per thousand range. Detection limits of parts per million (ppm) are possible, but require special conditions: concentration at top surface or very long collection time (overnight).
- XPS is routinely used to analyze inorganic compounds, metal alloys, semiconductors, polymers, elements, catalysts, glasses, ceramics, paints, papers, inks, woods, plant parts, make-up, teeth, bones, medical implants, bio-materials, viscous oils, glues, ion modified materials and many others.

XPS is used to measure**:**

- elemental composition of the surface (top 1–10 nm usually);

- empirical formula of pure materials;
- elements that contaminate a surface;
- chemical or electronic state of each element in the surface;
- uniformity of elemental composition across the top surface (or line profiling or mapping);
- uniformity of elemental composition as a function of ion beam etching (or depth profiling).

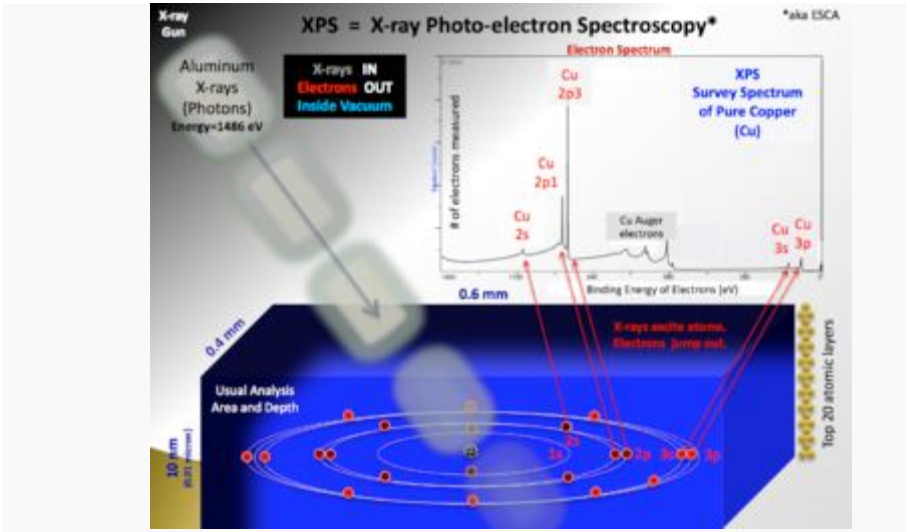


Fig. 7 Schematic of XPS physics - Photoelectric Effect.

XPS can be performed using either a commercially built XPS system, a privately built XPS system or a synchrotron-based light source combined with a custom designed electron analyzer. Commercial XPS instruments in the year 2005 used either a highly focused 20 to 200 micrometer beam of monochromatic aluminium Kα X-rays or a broad 10–30 mm beam of non-monochromatic (polychromatic) magnesium X-rays. A few, special design, XPS instruments can analyze volatile liquids or gases, materials at low or high temperatures or materials at roughly 1 torr vacuum, but there are relatively few of these types of XPS systems.

Because the energy of an X-ray with particular wavelength is known, the electron binding energy of each of the emitted electrons can be determined by using an equation that is based on the work of Ernest Rutherford (1914):

$$E_{binding} = E_{photon} - (E_{kinetic} + \varphi)$$

where $E_{binding}$ is the binding energy (BE) of the electron, E_{photon} is the energy of the X-ray photons being used, $E_{kinetic}$ is the kinetic energy of the electron as measured by the instrument and φ is the work function of the spectrometer (not the material).

7.1 Physics

A typical XPS spectrum is a plot of the number of electrons detected (sometimes per unit time) (Y-axis, ordinate) versus the binding energy of the electrons detected (X-axis, abscissa). Each element produces a characteristic set of XPS peaks at characteristic binding energy values that directly identify each element that exist in or on the surface of the material being analyzed. These characteristic peaks correspond to the electron configuration of the electrons within the atoms, e.g., 1s, 2s, 2p, 3s, etc. The number of detected electrons in each of the characteristic peaks is directly related to the amount of element within the area (volume) irradiated. To generate atomic percentage values, each raw XPS signal must be corrected by dividing its signal intensity (number of electrons detected) by a "relative sensitivity factor" (RSF) and normalized over all of the elements detected.

To count the number of electrons at each kinetic energy value, with the minimum of error, XPS must be performed under ultra-high vacuum (UHV) conditions because electron counting detectors in XPS instruments are typically one meter away from the material irradiated with X-rays.

It is important to note that XPS detects only those electrons that have actually escaped into the vacuum of the instrument. The photo-emitted electrons that have escaped into the vacuum of the instrument are those that originated from within the top 10 to 12 nm of the material. All of the deeper photo-emitted electrons, which were generated as the X-rays penetrated 1–5 micrometers of the material, are either recaptured or trapped in various excited states within the material. For most applications, it is, in effect, a non-destructive technique that measures the surface chemistry of any material.

7.2 Components of an XPS System

The main components of a commercially made XPS system include:

- A source of X-rays;
- An ultra-high vacuum (UHV) stainless steel chamber with UHV pumps;

- An electron collection lens;
- An electron energy analyzer;
- Mu-metal magnetic field shielding;
- An electron detector system;
- A moderate vacuum sample introduction chamber;
- Sample mounts;
- A sample stage;
- A set of stage manipulators.

Monochromatic aluminium K-alpha X-rays are normally produced by diffracting and focusing a beam of non-monochromatic X-rays off of a thin disc of natural, crystalline quartz with a <1010> orientation. The resulting wavelength is 8.3386 angstroms (0.83386 nm) which corresponds to photon energy of 1486.7 eV. The energy width of the monochromated X-rays is 0.16 eV, but the common electron energy analyzer (spectrometer) produces an ultimate energy resolution on the order of 0.25 eV which, in effect, is the ultimate energy resolution of most commercial systems. When working under practical, everyday conditions, high-energy-resolution settings will produce peak widths (FWHM) between 0.4–0.6 eV for various pure elements and some compounds.

Non-monochromatic magnesium X-rays have a wavelength of 9.89 angstroms (0.989 nm) which corresponds to photon energy of 1253 eV. The energy width of the non-monochromated X-ray is roughly 0.70 eV, which, in effect is the ultimate energy resolution of a system using non-monochromatic X-rays. Non-monochromatic X-ray sources do not use any crystals to diffract the X-rays which allow all primary X-rays lines and the full range of high-energy Bremsstrahlung X-rays (1–12 keV) to reach the surface. The typical ultimate high-energy-resolution (FWHM) when using this source is 0.9–1.0 eV, which includes with the spectrometer-induced broadening, pass-energy settings and the peak-width of the non-monochromatic magnesium X-ray source.

7.3 Peak Identification

The number of peaks produced by a single element varies from 1 to more than 20. Tables of binding energies (BEs) that identify the shell and spin-orbit of each peak produced by a given element are included with modern XPS instruments, and can be found in various handbooks and websites. Because these experimentally determined BEs are characteristic of

specific elements, they can be directly used to identify experimentally measured peaks of a material with unknown elemental composition.

Before beginning the process of peak identification, the analyst must determine if the BEs of the unprocessed survey spectrum (0-1400 eV) have or have not been shifted due to a positive or negative surface charge. This is most often done by looking for two peaks that due to the presence of carbon and oxygen.

7.4 Charge Referencing Insulators

Charge referencing is needed when a sample suffers either a positive (+) or negative (-) charge induced shift of experimental BEs. Charge referencing is needed to obtain meaningful BEs from both wide-scan, high sensitivity (low energy resolution) survey spectra (0-1100 eV), and also narrow-scan, chemical state (high energy resolution) spectra.

Charge induced shifting causes experimentally measured BEs of XPS peaks to appear at BEs that are greater or smaller than true BEs. Charge referencing is performed by adding or subtracting a "Charge Correction Factor" to each of the experimentally measured BEs. In general, the BE of the hydrocarbon peak of the C (1s) XPS signal is used to charge reference (charge correct) all BEs obtained from non-conductive (insulating) samples or conductors that have been deliberately insulated from the sample mount.

Charge induced shifting is normally due to: a modest excess of low voltage (-1 to -20 eV) electrons attached to the surface, or a modest shortage of electrons (+1 to +15 eV) within the top 1-12 nm of the sample caused by the loss of photo-emitted electrons. The degree of charging depends on various factors. If, by chance, the charging of the surface is excessively positive, then the spectrum might appear as a series of rolling hills, not sharp peaks as shown in the example spectrum.

The C (1s) BE of the hydrocarbon species (moieties) of the "Adventitious" carbon that appears on all, air-exposed, conductive and semiconductive materials is normally found between 284.5 eV and 285.5 eV. For convenience, the C (1s) of hydrocarbon moieties is defined to appear between 284.6 eV and 285.0 eV. A value of 284.8 eV has become popular in recent years. However, some recent reports indicate that 284.9 eV or 285.0 eV represents hydrocarbons attached on metals, not the natural native oxide. The 284.8 eV BE is routinely used as the "Reference BE" for charge referencing insulators. When the C (1s) BE is used for charge referencing,

then the charge correction factor is the difference between 284.8 eV and the experimentally measured C (1s) BE of the hydrocarbon moieties.

When using a monochromatic XPS system together with a low voltage electron flood gun for charge compensation the experimental BEs of the C (1s) hydrocarbon peak is often 4-5 eV smaller than the reference BE value (284.8 eV). In this case, all experimental BEs appear at lower BEs than expected and need to be increased by adding a value ranging from 4 to 5 eV. Non-monochromatic XPS systems are not usually equipped with a low voltage electron flood gun so the BEs will normally appear at higher BEs than expected. It is normal to subtract a charge correction factor from all BEs produced by a non-monochromatic XPS system.

Conductive materials and most native oxides of conductors should never need charge referencing. Conductive materials should never be charge referenced unless the topmost layer of the sample has a thick non-conductive film.

7.5 Peak-Fitting

The process of peak-fitting high energy resolution XPS spectra is still a mixture of art, science, knowledge and experience. The peak-fit process is affected by instrument design, instrument components, experimental settings (aka analysis conditions) and sample variables. Most instrument parameters are constant while others depend on the choice of experimental settings.

Before starting any peak-fit effort, the analyst performing the peak-fit needs to know if the topmost 15 nm of the sample is expected to be a homogeneous material or is expected to be a mixture of materials. If the top 15 nm is a homogeneous material with only very minor amounts of adventitious carbon and adsorbed gases, then the analyst can use theoretical peak area ratios to enhance the peak-fitting process.

Variables that affect or define peak-fit results include:

- FWHMs;
- Chemical Shifts;
- Peakshapes;
- Instrument design factors;
- Experimental settings;
- Sample factors.

7.6 Application

XPS is routinely used to determine:

- What elements and the quantity of those elements that are present within the top 1-12 nm of the sample surface.
- What contamination, if any, exists in the surface or the bulk of the sample.
- Empirical formula of a material that is free of excessive surface contamination.
- The chemical state identification of one or more of the elements in the sample.
- The binding energy of one or more electronic states.
- The thickness of one or more thin layers (1–8 nm) of different materials within the top 12 nm of the surface.
- The density of electronic states.

8. UV and VISIBLE SPECTROSCOPY

Ultraviolet–Visible Spectroscopy or **Ultraviolet-Visible Spectrophotometry** (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO) the higher the wavelength of light it can absorb.

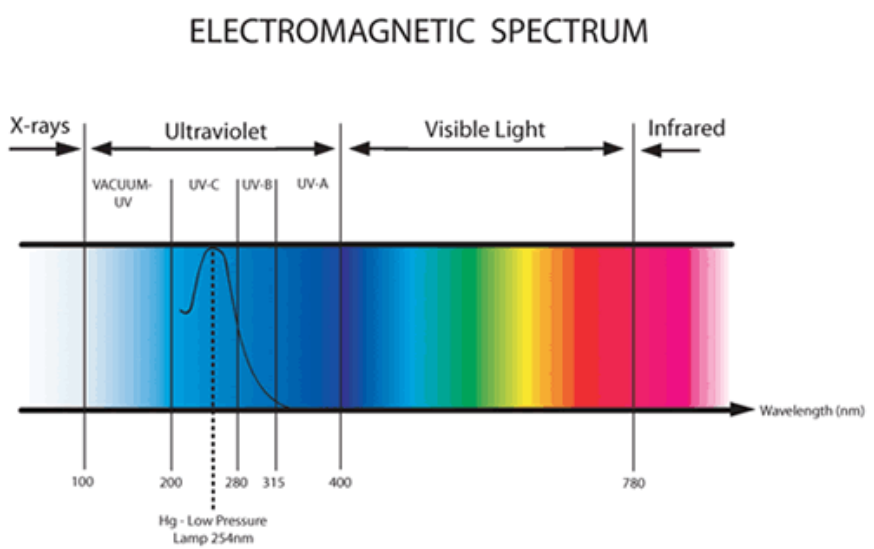


Fig. 8 Electromagnetic spectrum.

8.1 Beer-Lambert Law

The method is most often used in a quantitative way to determine concentrations of an absorbing species in solution, using the Beer-Lambert law:

$$A = log_{10}(I_0/I) = \epsilon \cdot c \cdot L,$$

where A is the measured absorbance, I_0 is the intensity of the incident light at a given wavelength, I is the transmitted intensity, L the pathlength through the sample, and c the concentration of the absorbing species. For each species and wavelength, ϵ is a constant known as the molar absorptivity or extinction coefficient. This constant is a fundamental molecular property in a given solvent, at a particular temperature and pressure, and has units of $1/M \cdot cm$ or often $AU/M \cdot cm$.

The absorbance and extinction ϵ are sometimes defined in terms of the natural logarithm instead of the base-10 logarithm.

The Beer-Lambert Law is useful for characterizing many compounds but does not hold as a universal relationship for the concentration and absorption of all substances. A 2nd order polynomial relationship between absorption and concentration is sometimes encountered for very large, complex molecules such as organic dyes (Xylenol Orange or Neutral Red, for example).

Practical Considerations

The Beer-Lambert law has implicit assumptions that must be met experimentally for it to apply. For instance, the chemical makeup and physical environment of the sample can alter its extinction coefficient. The chemical and physical conditions of a test sample therefore must match reference measurements for conclusions to be valid.

Spectral Bandwidth

A given spectrometer has a spectral bandwidth that characterizes how monochromatic the light is. If this bandwidth is comparable to the width of the absorption features, then the measured extinction coefficient will be altered. In most reference measurements, the instrument bandwidth is kept below the width of the spectral lines. When a new material is being measured, it may be necessary to test and verify if the bandwidth is sufficiently narrow. Reducing the spectral bandwidth will reduce the energy passed to the detector and will, therefore, require a longer measurement time to achieve the same signal to noise ratio.

Wavelength Error

In liquids, the extinction coefficient usually changes slowly with wavelength. A peak of the absorbance curve (a wavelength where the absorbance reaches a maximum) is where the rate of change in absorbance with wavelength is smallest. Measurements are usually made at a peak to minimize errors produced by errors in wavelength in the instrument, that is errors due to having a different extinction coefficient than assumed.

Stray Light

Another important factor is the purity of the light used. The most important factor affecting this is the stray light level of the monochromator. The detector used is broadband, it responds to all the light that reaches it. If a significant amount of the light passed through the sample contains wavelengths that have much lower extinction coefficients than the nominal one, the instrument will report an incorrectly low absorbance. Any instrument will reach a point where an increase in sample concentration will not result in an increase in the reported absorbance, because the detector is simply responding to the stray light. In practice the concentration of the sample or the optical path length must be adjusted to place the unknown absorbance within a range that is valid for the instrument. Sometimes an empirical calibration function is developed, using known concentrations of the sample, to allow measurements into the region where the instrument is becoming nonlinear.

As a rough guide, an instrument with a single monochromator would typically have a stray light level corresponding to about 3 AU, which would make measurements above about 2 AU problematic. A more complex instrument with a double monochromator would have a stray light level corresponding to about 6 AU, which would therefore allow measuring a much wider absorbance range.

Absorption Flattening

At sufficiently high concentrations, the absorption bands will saturate and show absorption flattening. The absorption peak appears to flatten because close to 100% of the light is already being absorbed. The concentration at which this occurs depends on the particular compound being measured. One test that can be used to test for this effect is to vary the path length of the measurement. In the Beer-Lambert law, varying concentration and path length has an equivalent effect—diluting a solution by a factor of 10 has the same effect as shortening the path length by a factor of 10. If cells of different path lengths are available, testing if this relationship holds true is one way to judge if absorption flattening is occurring.

Solutions that are not homogeneous can show deviations from the Beer-Lambert law because of the phenomenon of absorption flattening. This can happen, for instance, where the absorbing substance is located within suspended particles. The deviations will be most noticeable under conditions of low concentration and high absorbance. The reference describes a way to correct for this deviation.

Measurement Uncertainty Sources

The above factor contribute to the measurement uncertainty of the results obtained with UV/Vis spectrophotometry. If UV/Vis spectrophotometry is used in quantitative chemical analysis then the results are additionally affected by uncertainty sources arising from the nature of the compounds and/or solutions that are measured. These include spectral interferences caused by absorption band overlap, fading of the color of the absorbing species (caused by decomposition or reaction) and possible composition mismatch between the sample and the calibration solution.

8.2 Ultraviolet-Visible Spectrometer

The instrument used in ultraviolet-visible spectroscopy is called a UV/Vis spectrophotometer. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I_0). The ratio I/I_0 is called the transmittance, and is usually expressed as a percentage (%T). The absorbance, A, is based on the transmittance:

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

The UV-visible spectrophotometer can also be configured to measure reflectance. In this case, the spectrophotometer measures the intensity of light reflected from a sample (I), and compares it to the intensity of light reflected from a reference material (I_0)(such as a white tile). The ratio I/I_0 is called the reflectance, and is usually expressed as a percentage (%R).

The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light, and a detector. The radiation source is often a Tungsten filament (300-2500 nm), a deuterium arc lamp, which is continuous over the ultraviolet region (190-400 nm), Xenon arc lamps, which is continuous from 160-2,000 nm; or more recently, light emitting diodes (LED) for the visible wavelengths. The detector is typically a photomultiplier tube, a photodiode, a photodiode array or a charge-coupled device (CCD). Single photodiode detectors and photomultiplier tubes are used with scanning monochromators, which filter the light so that only light of a single wavelength reaches the detector at one time. The scanning monochromator moves the diffraction grating to "step-through" each wavelength so that its intensity may be measured as a function of wavelength. Fixed monochromators are used with CCDs and photodiode arrays. As both of these devices consist of many detectors grouped into one or two dimensional arrays, they are able to collect light of different wavelengths on different pixels or groups of pixels simultaneously.

A spectrophotometer can be either single beam or double beam. In a single beam instrument, all of the light passes through the sample cell. I_0 must be measured by removing the sample. This was the earliest design, but is still in common use in both teaching and industrial labs.

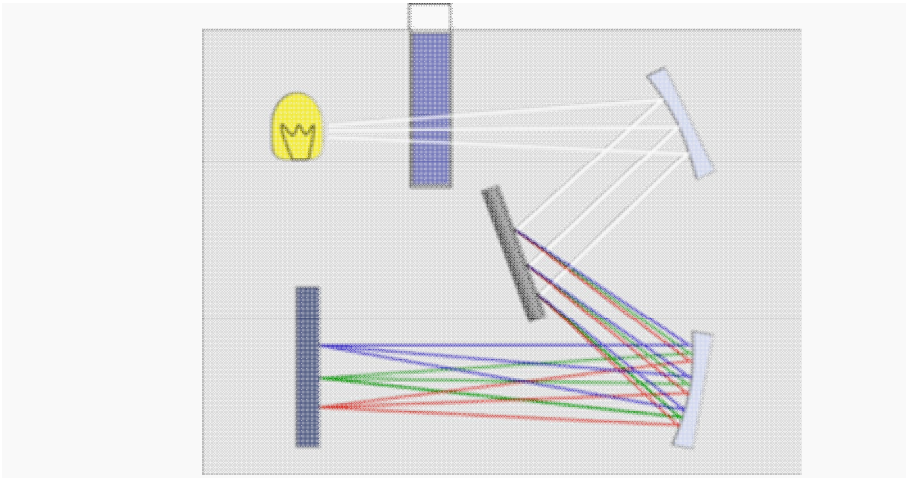


Fig. 9 Diagram of a single-beam UV/Vis spectrophotometer.

In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission (or 0 Absorbance), and the measurement displayed is the ratio of the two beam intensities. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam in synchronism with the chopper. There may also be one or more dark intervals in the chopper cycle. In this case, the measured beam intensities may be corrected by subtracting the intensity measured in the dark interval before the ratio is taken.

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm. (This width becomes the path length, L, in the Beer-Lambert law.) Test tubes can also be used as cuvettes in some instruments. The type of sample container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions. Glass and plastic cuvettes are also

common, although glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.

Specialized instruments have also been made. These include attaching spectrophotometers to telescopes to measure the spectra of astronomical features. UV-visible microspectrophotometers consist of a UV-visible microscope integrated with a UV-visible spectrophotometer.

A complete spectrum of the absorption at all wavelengths of interest can often be produced directly by a more sophisticated spectrophotometer. In simpler instruments the absorption is determined one wavelength at a time and then compiled into a spectrum by the operator. By removing the concentration dependence, the extinction coefficient (ϵ) can be determined as a function of wavelength.

8.3 Application

UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metalions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

- Solutions of transition metal ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λ_{max}).
- Organic compounds, especially those with a high degree of conjugation (e.g. DNA, RNA, protein), also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble compounds, or ethanol for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths.) Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases.
- While charge transfer complexes also give rise to colours, the colours are often too intense to be used for quantitative measurement.

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

A UV/Vis spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response assumed to be proportional to the concentration. For accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; this is very similar to the use of calibration curves. The response (e.g., peak height) for a particular concentration is known as the response factor.

The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule. The Woodward-Fieser rules, for instance, are a set of empirical observations used to predict λ_{max}, the wavelength of the most intense UV/Vis absorption, for conjugated organic compounds such as dienes and ketones. The spectrum alone is not, however, a specific test for any given sample. The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum. Experimental variations such as the slit width (effective bandwidth) of the spectrophotometer will also alter the spectrum. To apply UV/Vis spectroscopy to analysis, these variables must be controlled or accounted for in order to identify the substances present.

9. INFRARED SPECTROSCOPY

Infrared Spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer.

The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far- infrared, named for their

relation to the visible spectrum. The higher-energy near-IR, approximately 14000–4000 cm^{-1} (0.8–2.5 μm wavelength) can excite overtone or harmonic vibrations. The mid-infrared, approximately 4000–400 cm^{-1} (2.5–25 μm) may be used to study the fundamental vibrations and associated rotational-vibrational structure. The far-infrared, approximately 400–10 cm^{-1} (25–1000 μm), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The names and classifications of these subregions are conventions, and are only loosely based on the relative molecular or electromagnetic properties.

Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates. The energies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling.

In particular, in the Born–Oppenheimer and harmonic approximations, i.e. when the molecular Hamiltonian corresponding to the electronic ground state can be approximated by a harmonic oscillator in the neighborhood of the equilibrium molecular geometry, the resonant frequencies are determined by the normal modes corresponding to the molecular electronic ground state potential energy surface. Nevertheless, the resonant frequencies can be in a first approach related to the strength of the bond, and the mass of the atoms at either end of it. Thus, the frequency of the vibrations can be associated with a particular bond type.

In order for a vibrational mode in a molecule to be "IR active," it must be associated with changes in the dipole. A permanent dipole is not necessary, as the rule requires only a change in dipole moment.

A molecule can vibrate in many ways, and each way is called a vibrational mode. For molecules with N atoms in them, linear molecules have $3N - 5$ degrees of vibrational modes, whereas nonlinear molecules have $3N - 6$ degrees of vibrational modes (also called vibrational degrees of freedom). As an example H_2O, a non-linear molecule, will have $3 \times 3 - 6 = 3$ degrees of vibrational freedom, or modes.

Simple diatomic molecules have only one bond and only one vibrational band. If the molecule is symmetrical, e.g. N_2, the band is not observed in the IR spectrum, but only in the Raman spectrum. Asymmetrical diatomic molecules, e.g. CO, absorb in the IR spectrum. More complex molecules have many bonds, and their vibrational spectra are correspondingly more complex, i.e. big molecules have many peaks in their IR spectra.

The atoms in a CH_2 group, commonly found in organic compounds, can vibrate in six different ways: symmetric and antisymmetric stretching, scissoring, rocking, wagging and twisting.

The simplest and most important IR bands arise from the "normal modes", the simplest distortions of the molecule. In some cases, "overtone bands" are observed. These bands arise from the absorption of a photon that leads to a doubly excited vibrational state. Such bands appear at approximately twice the energy of the normal mode. Some vibrations, so-called "combination modes", involve more than one normal mode. The phenomenon of Fermi resonance can arise when two modes are similar in energy, Fermi resonance results in an unexpected shift in energy and intensity of the bands.

The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond, absorption occurs. Examination of the transmitted light reveals how much energy was absorbed at each frequency (or wavelength). This can be achieved by scanning the wavelength range using a monochromator. Alternatively, the whole wavelength range is measured at once using a Fourier transform instrument and then a transmittance or absorbance spectrum is generated using a dedicated procedure. Analysis of the position, shape and intensity of peaks in this spectrum reveals details about the molecular structure of the sample.

This technique works almost exclusively on samples with covalent bonds. Simple spectra are obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra. The technique has been used for the characterization of very complex mixtures. Spectra issues with Infrared Flourescense are rare.

9.1 Sample Preparation

Gaseous samples require a sample cell with a long pathlength to compensate for the diluteness. The pathlength of the sample cell depends on the concentration of the compound of the interest. A simple glass tube with length of 5 to 10 cm equipped with infrared windows at the both ends of the tube can be used for concentrations down to several hundred ppms. Sample gas concentrations well below ppm can be measured with a White's cell in

which the infrared light is guided with mirrors to travel through the gas. White's cells are available with optical pathlength starting from 0.5 m up to hundred meters.

Liquid samples can be sandwiched between two plates of a salt (commonly sodium chloride, or common salt, although a number of other salts such as potassium bromide or calcium fluoride are also used). The plates are transparent to the infrared light and do not introduce any lines onto the spectra.

Solid samples can be prepared in a variety of ways. One common method is to crush the sample with an oily mulling agent (usually Nujol) in a marble or agate mortar, with a pestle. A thin film of the mull is smeared onto salt plates and measured. The second method is to grind a quantity of the sample with a specially purified salt (usually potassium bromide) finely (to remove scattering effects from large crystals). This powder mixture is then pressed in a mechanical press to form a translucent pellet through which the beam of the spectrometer can pass. A third technique is the "cast film" technique, which is used mainly for polymeric materials. The sample is first dissolved in a suitable, non hygroscopic solvent. A drop of this solution is deposited on surface of KBr or NaCl cell. The solution is then evaporated to dryness and the film formed on the cell is analysed directly. Care is important to ensure that the film is not too thick otherwise light cannot pass through. This technique is suitable for qualitative analysis. The final method is to use microtomy to cut a thin (20–100 μm) film from a solid sample. This is one of the most important ways of analysing failed plastic products for example because the integrity of the solid is preserved.

In photoacoustic spectroscopy the need for sample treatment is minimal. The sample, liquid or solid, is placed into the sample cup which is inserted into the photoacoustic cell which is then sealed for the measurement. The sample may be one solid piece, powder or basically in any form for the measurement. For example, a piece of rock can be inserted into the sample cup and the spectrum measured from it.

It is important to note that spectra obtained from different sample preparation methods will look slightly different from each other due to differences in the samples' physical states.

9.2 Usual IR Spectrometer Device

To take the infrared spectrum of a sample, it is necessary to measure both the sample and a "reference" (or "control"). This is because each

measurement is affected by not only the light-absorption properties of the sample, but also the properties of the instrument (for example, what light source is used, what infrared detector is used, etc.). The reference measurement makes it possible to eliminate the instrument influence. Mathematically, the sample transmission spectrum is divided by the reference transmission spectrum.

The appropriate "reference" depends on the measurement and its goal. The simplest reference measurement is to simply remove the sample (replacing it by air). However, sometimes a different reference is more useful. For example, if the sample is a dilute solute dissolved in water in a beaker, then a good reference measurement might be to measure pure water in the same beaker. Then the reference measurement would cancel out not only all the instrumental properties (like what light source is used), but also the light-absorbing and light-reflecting properties of the water and beaker, and the final result would just show the properties of the solute (at least approximately).

A common way to compare to a reference is sequentially: first measure the reference, then replace the reference by the sample and measure the sample. This technique is not perfectly reliable; if the infrared lamp is a bit brighter during the reference measurement, then a bit dimmer during the sample measurement, the measurement will be distorted. More elaborate methods, such as a "two-beam" setup (see Fig. 10), can correct for these types of effects to give very accurate results. The Standard addition method can be used to statistically cancel these errors.

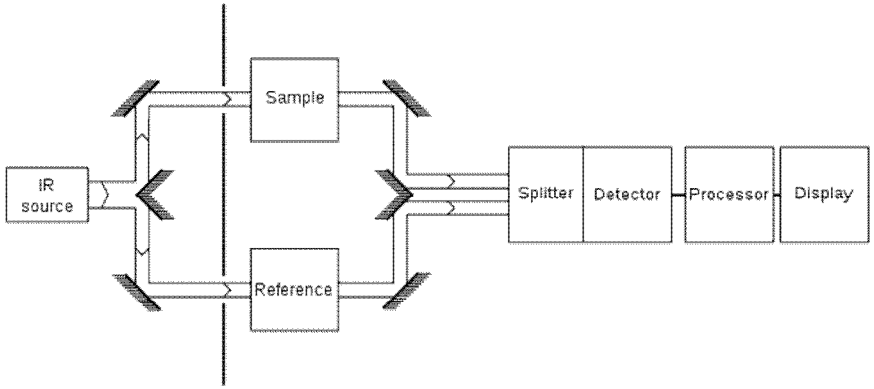


Fig. 10 Schematics of a two-beam absorption spectrometer.

A beam of infrared light is produced, passed through an interferometer, and then split into two separate beams. One is passed through the sample, the other passed through a reference. The beams are both reflected back towards a detector, however first they pass through a splitter, which quickly alternates which of the two beams enters the detector. The two signals are then compared and a printout is obtained. This "two-beam" setup gives accurate spectra even if the intensity of the light source drifts over time.

9.3 IR Spectrometer with Fourier Transforms

Fourier Transform Infrared (FTIR) Spectroscopy is a measurement technique that allows one to record infrared spectra. Infrared light is guided through aninterferometer and then through the sample (or vice versa). A moving mirror inside the apparatus alters the distribution of infrared light that passes through the interferometer. The signal directly recorded, called an "interferogram", represents light output as a function of mirror position. A data-processing technique called Fourier transform turns this raw data into the desired result (the sample's spectrum): Light output as a function of infrared wavelength (or equivalently, wave number). As described above, the sample's spectrum is always compared to a reference.

There is an alternate method for taking spectra (the "dispersive" or "scanning monochromator" method), where one wavelength at a time passes through the sample. The dispersive method is more common in UV-Vis spectroscopy, but is less practical in the infrared than the FTIR method. One reason that FTIR is favored is called "Fellgett's advantage" or the "multiplex advantage": the information at all frequencies is collected simultaneously, improving both speed and signal-to-noise ratio. Another is called "Jacquinot's Throughput Advantage": a dispersive measurement requires detecting much lower light levels than an FTIR measurement. There are other advantages, as well as some disadvantages, but virtually all modern infrared spectrometers are FTIR instruments.

9.4 Radiation Sources

As a source of radiation in the infrared region are used hot solids. For these sources, the intensity distribution of radiation by wavelengths depends on temperature and is described by Planck's radiation law. This distribution is irregularly and has a distinct maximum. For IR spectroscopy is necessary to cut off an intense short-wave radiation in the visible region and leave a long-wavelength and relatively less intense radiation usually in the region of 4000-400 cm^{-1}.

The most common sources of infrared radiation is Nernst pin, made of yttrium and zirconium oxides, and silicon carbide globar. For Nernst pin the working temperature is about 1900°C, for globar - about 1350°C. Less intense, but longer in operation sources are made of refractory alloys (for example, chromium or nickel). They are heated to a temperature of 800°C.

For the far-infrared (from 200 to 10 cm^{-1}) is necessary to use special radiation sources. Most often is used high pressure mercury discharge lamps.

In the near-infrared region (4000-12800 cm^{-1}) can be used ordinary tungsten filament lamp.

9.5 Detectors

As detectors (receivers) are used IR thermal detectors – bolometers and thermocouples. Thermocouple converts the energy of infrared radiation into thermal and then into electricity. Occurring as a result of this process, the potential difference is recorded in the usual way. The bolometer operates on the principle of the resistance thermometer. Bolometer working material is a metal or alloy (platinum, nickel, as well as semiconductor materials), the electrical resistance of which varies strongly with temperature.

A general problem of measuring the intensity of infrared radiation is the presence of a significant thermal noise of the environment with a relatively small useful signal. Detectors of infrared radiation should be as much as possible isolated from the environment. In addition, there is used a modulation signal with the chopper to distinguish it from thermal noise.

9.6 Applications

Infrared spectroscopy is a simple and reliable technique widely used in both organic and inorganic chemistry, in research and industry. It is used in quality control, dynamic measurement, and monitoring applications such as the long-term unattended measurement of CO_2 concentrations in greenhouses and growth chambers by infrared gas analyzers.

It is also used in forensic analysis in both criminal and civil cases, for example in identifying polymer degradation. It can be used in detecting how much alcohol is in the blood of a suspected drunk driver measured as 1/10,000 g/mL = 100 μg/mL.

A useful way of analysing solid samples without the need for cutting samples uses attenuated total reflectance (or ATR) spectroscopy. Using this approach, samples are pressed against the face of a single crystal. The infrared radiation passes through the crystal and only interacts with the sample at the interface between the two materials.

With increasing technology in computer filtering and manipulation of the results, samples in solution can now be measured accurately (water produces a broad absorbance across the range of interest, and thus renders the spectra unreadable without this computer treatment).

Some instruments will also automatically tell you what substance is being measured from a store of thousands of reference spectra held in storage.

Infrared spectroscopy is also useful in measuring the degree of polymerization in polymer manufacture. Changes in the character or quantity of a particular bond are assessed by measuring at a specific frequency over time. Modern research instruments can take infrared measurements across the range of interest as frequently as 32 times a second. This can be done whilst simultaneous measurements are made using other techniques. This makes the observations of chemical reactions and processes quicker and more accurate.

Infrared spectroscopy has also been successfully utilized in the field of semiconductor microelectronics: for example, infrared spectroscopy can be applied to semiconductors like silicon, gallium arsenide, gallium nitride, zinc selenide, amorphous silicon, silicon nitride, etc.

The instruments are now small, and can be transported, even for use in field trials.

10. NUCLEAR MAGNETIC RESONANCE

Nuclear Magnetic Rresonance (**NMR**) was first described and measured in molecular beams by Isidor Rabi in 1938, and in 1944, Rabi was awarded the Nobel Prize in physics for this work. In 1946, Felix Bloch and Edward Mills Purcell expanded the technique for use on liquids and solids, for which they shared the Nobel Prize in Physics in 1952.

Nuclear magnetic resonance is a physical phenomenon in which magnetic nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope

of the atoms; in practical applications, the frequency is similar to VHF and UHF television broadcasts (60–1000 MHz). NMR allows the observation of specific quantum mechanical magnetic properties of the atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals, and non-crystalline materials through NMR spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).

All isotopes that contain an odd number of protons and/or of neutrons have an intrinsic magnetic moment and angular momentum, in other words a nonzero spin, while all nuclides with even numbers of both have a total spin of zero. The most commonly studied nuclei are ^{1}H and ^{13}C, although nuclei from isotopes of many other elements (e.g. ^{2}H, ^{6}Li, ^{10}B, ^{11}B, ^{14}N, ^{15}N, ^{17}O, ^{19}F, ^{23}Na, ^{29}Si, ^{31}P, ^{35}Cl, ^{113}Cd, ^{129}Xe, ^{195}Pt) have been studied by high-field NMR spectroscopy as well.

A key feature of NMR is that the resonance frequency of a particular substance is directly proportional to the strength of the applied magnetic field. It is this feature that is exploited in imaging techniques; if a sample is placed in a non-uniform magnetic field then the resonance frequencies of the sample's nuclei depend on where in the field they are located. Since the resolution of the imaging technique depends on the magnitude of magnetic field gradient, many efforts are made to develop increased field strength, often using superconductors. The effectiveness of NMR can also be improved using hyperpolarization, and/or using two-dimensional, three-dimensional and higher-dimensional multi-frequency techniques.

The principle of NMR usually involves two sequential steps:

- The alignment (polarization) of the magnetic nuclear spins in an applied, constant magnetic field H_0.
- The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field (H_0) and the nuclei of observation.

The two fields are usually chosen to be perpendicular to each other as this maximizes the NMR signal strength. The resulting response by the total magnetization (M) of the nuclear spins is the phenomenon that is exploited in NMR spectroscopy and magnetic resonance imaging. Both use intense applied magnetic fields (H_0) in order to achieve dispersion and very high stability to deliver spectral resolution, the details of which are described by chemical shifts, the Zeeman effect, and Knight shifts (in metals).

NMR phenomena are also utilized in low-field NMR, NMR spectroscopy and MRI in the Earth's magnetic field (referred to as Earth's field NMR), and in several types of magnetometers.

10.1 Nuclear Spin and Magnets

All nucleons, that are neutrons and protons, composing any atomic nucleus, have the intrinsic quantum property of spin. The overall spin of the nucleus is determined by the spin quantum number *S*. If the number of both the protons and neutrons in a given nuclide are even then $S = 0$, i.e. there is no overall spin. Then, just as electrons pair up in atomic orbitals, so do even numbers of protons or even numbers of neutrons (which are also spin-$\frac{1}{2}$ particles and hence fermions) pair up giving zero overall spin.

However, a proton and neutron will have lower energy when their spins are parallel, not anti-parallel, since this parallel spin alignment does not infringe upon the Pauli Exclusion Principle, but instead it has to do with the quark structure of these two nucleons. Therefore, the spin ground state for the deuteron (the deuterium nucleus, or the ^{2}H isotope of hydrogen) that has only a proton and a neutron corresponds to a spin value of 1, not of zero. The single, isolated deuteron therefore exhibits an NMR absorption spectrum characteristic of a quadrupolar nucleus of spin 1, which in the "rigid" state at very low temperatures is a characteristic ('Pake') doublet, (not a singlet as for a single, isolated ^{1}H, or any other isolated fermion or dipolar nucleus of spin 1/2). On the other hand, because of the Pauli Exclusion Principle, the tritium isotope of hydrogen must have a pair of anti-parallel spin neutrons (of total spin zero for the neutron-spin pair), plus a proton of spin 1/2. Therefore, the character of the tritium nucleus is again magnetic dipolar, not quadrupolar like its non-radioactive deuteron neighbor and the tritium nucleus total spin value is again 1/2, just like for the simpler, abundant hydrogen isotope, ^{1}H nucleus (the proton). The NMR absorption (radio) frequency for tritium is however slightly higher than that of ^{1}H because the tritium nucleus has a slightly higher gyromagnetic ratio than ^{1}H. In many other cases of non-radioactive nuclei, the overall spin is also non-zero. For example, the ^{27}Al nucleus has an overall spin value $S = \frac{5}{2}$.

A non-zero spin is thus always associated with a non-zero magnetic moment (μ) via the relation $\mu = \gamma S$, where γ is the gyromagnetic ratio. It is this magnetic moment that allows the observation of NMR absorption spectra caused by transitions between nuclear spin levels. Most nuclides (with some rare exceptions) that have both even numbers of protons and even

numbers of neutrons, also have zero nuclear magnetic moments, and they also have zero magnetic dipole and quadrupole moments. Hence, such nuclides do not exhibit any NMR absorption spectra. Thus, ^{18}O is an example of a nuclide that has no NMR absorption, whereas ^{13}C, ^{31}P, ^{35}Cl and ^{37}Cl are nuclides that do exhibit NMR absorption spectra. The last two nuclei are quadrupolar nuclei whereas the preceding two nuclei (^{13}C and ^{31}P) are dipolar ones.

Electron spin resonance (ESR) is a related technique in which transitions between electronic spin levels are detected rather than nuclear ones. The basic principles are similar but the instrumentation, data analysis, and detailed theory are significantly different. Moreover, there is a much smaller number of molecules and materials with unpaired electron spins that exhibit ESR (or electron paramagnetic resonance (EPR)) absorption than those that have NMR absorption spectra. ESR has much higher sensitivity than NMR does.

10.2 Values of Spin Angular Momentum

The angular momentum associated with nuclear spin is quantized. This means both that the magnitude of angular momentum is quantized (i.e. *S* can only take on a restricted range of values), and also that the orientation of the associated angular momentum is quantized. The associated quantum number is known as the magnetic quantum number, *m*, and can take values from $+S$ to $-S$, in integer steps. Hence for any given nucleus, there is a total of $2S + 1$ angular momentum states.

The z-component of the angular momentum vector (S) is therefore $S_z = m\hbar$, where $\hbar$ is the reduced Planck constant. The z-component of the magnetic moment is simply:

$$\mu_z = \gamma S_z = \gamma m \hbar$$

10.3 Spin Behavior in a Magnetic Field

Consider nuclei which have a spin of one-half, like ^{1}H, ^{13}C or ^{19}F. The nucleus has two possible spin states: $m = \frac{1}{2}$ or $m = -\frac{1}{2}$ (also referred to as spin-up and spin-down, or sometimes α and β spin states, respectively). These states are degenerate that is they have the same energy. Hence the number of atoms in these two states will be approximately equal at thermal equilibrium.

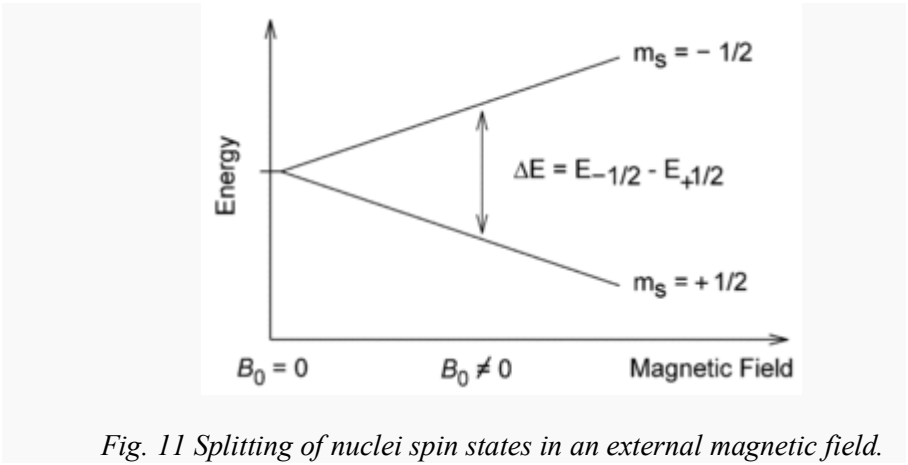


Fig. 11 Splitting of nuclei spin states in an external magnetic field.

If a nucleus is placed in a magnetic field, however, the interaction between the nuclear magnetic moment and the external magnetic field mean the two states no longer have the same energy. The energy of a magnetic moment μ when in a magnetic field B_0 is given by:

$$E = -\mu \cdot B_0 = -\mu_x B_{0x} - \mu_y B_{0y} - \mu_z B_{0z}$$

Usually the *z* axis is chosen to be along B_0, and the above expression reduces to:

$$E = -\mu_z B_0 ,$$

or alternatively:

$$E = -\gamma m \hbar B_0 .$$

As a result the different nuclear spin states have different energies in a non-zero magnetic field. In hand-waving terms, we can talk about the two spin states of a spin ½ as being aligned either with or against the magnetic field. If γ is positive (true for most isotopes) then $m = ½$ is the lower energy state.

The energy difference between the two states is:

$$\Delta E = -\gamma \hbar B_0 ,$$

and this difference results in a small population bias toward the lower energy state.

10.4 Magnetic Resonance by Nuclei

Resonant absorption by nuclear spins will occur only when electromagnetic radiation of the correct frequency (e.g. equaling the Larmor precession rate) is being applied to match the energy difference between the nuclear spin levels in a constant magnetic field of the appropriate strength.

The energy of an absorbed photon is then $E = h\nu_0$, where ν_0 is the resonance radiofrequency that has to match (that is, it has to be equal to the Larmor precession frequency ν_L of the nuclear magnetization in the constant magnetic field B_0). Hence, a magnetic resonance absorption will only occur when $\Delta E = h\nu_0$, which is when $\nu_0 = \gamma B_0/(2\pi)$. Such magnetic resonance frequencies typically correspond to the radio frequency (or RF) range of the electromagnetic spectrum for magnetic fields up to ~20 T. It is this magnetic resonant absorption which is detected in NMR.

10.5 Nuclear Shielding

It might appear from the above that all nuclei of the same nuclide (and hence the same γ) would resonate at the same frequency. This is not the case. The most important perturbation of the NMR frequency for applications of NMR is the "shielding" effect of the surrounding shells of electrons. Electrons, similar to the nucleus, are also charged and rotate with a spin to produce a magnetic field opposite to the magnetic field produced by the nucleus. In general, this electronic shielding reduces the magnetic field at the nucleus (which is what determines the NMR frequency).

As a result, the energy gap is reduced, and the frequency required to achieve resonance is also reduced. This shift in the NMR frequency due to the electronic molecular orbital coupling to the external magnetic field is called chemical shift, and it explains why NMR is able to probe the chemical structure of molecules, which depends on the electron density distribution in the corresponding molecular orbitals. If a nucleus in a specific chemical group is shielded to a higher degree by a higher electron density of its surrounding molecular orbital, then its NMR frequency will be shifted "upfield" (that is, a lower chemical shift), whereas if it is less shielded by such surrounding electron density, then its NMR frequency will be shifted "downfield" (that is, a higher chemical shift).

Unless the local symmetry of such molecular orbitals is very high (leading to "isotropic" shift), the shielding effect will depend on the orientation of the molecule with respect to the external field (B_0). Insolid-state NMR spectroscopy, magic angle spinning is required to average out this orientation dependence in order to obtain values close to the average chemical shifts. This is unnecessary in conventional NMR investigations of molecules, since rapid "molecular tumbling" averages out the chemical shift anisotropy (CSA). In this case, the term "average" chemical shift (ACS) is used.

10.6 Relaxation

The process called population relaxation refers to nuclei that return to the thermodynamic state in the magnet. This process is also called T_1, "spin-lattice" or "longitudinal magnetic" relaxation, where T_1 refers to the mean time for an individual nucleus to return to its thermal equilibrium state of the spins. Once the nuclear spin population is relaxed, it can be probed again, since it is in the initial, equilibrium (mixed) state.

The precessing nuclei can also fall out of alignment with each other (returning the net magnetization vector to a non-precessing field) and stop producing a signal. This is called T_2 or transverse relaxation. Because of the difference in the actual relaxation mechanisms involved (for example, intermolecular vs. intra-molecular magnetic dipole-dipole interactions), T_1 is usually (except in rare cases) longer than T_2 (that is, slower spin-lattice relaxation, for example because of smaller dipole-dipole interaction effects). In practice, the value of T_2^* which is the actually observed decay time of the observed NMR signal, or free induction decay, (to 1/e of the initial amplitude immediately after the resonant RF pulse) - also depends on the static magnetic field inhomogeneity, which is quite significant. (There is also a smaller but significant contribution to the observed FID shortening from the RF inhomogeneity of the resonant pulse). In the corresponding FT-NMR spectrums meaning the Fourier transform of the free induction decay - the T_2^* time is inversely related to the width of the NMR signal in frequency units. Thus, a nucleus with a long T_2 relaxation time gives rise to a very sharp NMR peak in the FT-NMR spectrum for a very homogeneous ("well-shimmed") static magnetic field, whereas nuclei with shorter T_2 values give rise to broad FT-NMR peaks even when the magnet is shimmed well. Both T_1 and T_2 depend on the rate of molecular motions as well as the gyromagnetic ratios of both the resonating and their strongly interacting, next-neighbor nuclei that are not at resonance.

A Hahn echo decay experiment can be used to measure the dephasing time, as shown in the animation below. The size of the echo is recorded for different spacings of the two pulses. This reveals decoherence which is not refocused by the π pulse. In simple cases, an exponential decay is measured which is described by the T_2 time.

NMR spectroscopy is one of the principal techniques used to obtain physical, chemical, electronic and structural information about molecules due to either the chemical shift, Zeeman effect, or the Knight shift effect, or a combination of both, on the resonant frequencies of the nuclei present in

the sample. It is a powerful technique that can provide detailed information on the topology, dynamics and three-dimensional structure of molecules in solution and the solid state. Thus, structural and dynamic information is obtainable (with or without "magic angle" spinning (MAS)) from NMR studies of quadrupolar nuclei (that is, those nuclei with spin $S > ½$) even in the presence of magnetic "dipole-dipole" interaction broadening (or simply, dipolar broadening) which is always much smaller than the quadrupolar interaction strength because it is a magnetic vs. an electric interaction effect.

Additional structural and chemical information may be obtained by performing double-quantum NMR experiments for quadrupolar nuclei such as ^2H. Also, nuclear magnetic resonance is one of the techniques that has been used to design quantum automata, and also build elementary quantum computers.

10.7 NMR Spectrometer Diagram

The sample in the ampule is placed in a strong uniform magnetic field H_0 created by an electromagnet constant, while the coil is under the influence of continuous high-frequency low-power field H_1. In the case of a field sweep at constant frequency of the generator $\nu=\omega/2\pi$ *is* performed slow scan in the resonance region, gradually changing H_0. When reaching the resonance condition, i.e. when the value of H_0 satisfies the basic equation of NMR, occurs the absorption of radiation energy at given frequency, fixed by the deviation of the pen recorder.

10.8 The Classic Description of the NMR Conditions

Nuclear magnetic moments in the field of external magnet (usually H_0 = 103 - 104 Hz) are not just along the force lines, but processed (rotate) with angular velocity ω_0 with respect to the direction of H_0. To create a resonance condition, the sample is affected by an additional ac field $H_1 << H_0$, rotating in the plane perpendicular to the direction of H_0. At the same time on the magnetic moment acts the force moment μH_1, tending to increase the angle between μ and H_0. If the field H_1 was rotated at a speed of ω, different from ω_0 by the value or direction, it would cause only minor short-termperturbations of the precession. If rotation of the field H_1 is synchronous with the precession of μ, then there is a constant perturbing effect of rejecting the μ negative direction along the z axis. Deviation requires the

expenditure of a certain energy that comes from a field source H_1. This energy is recorded as a signal of resonant absorption.

Thus, the phenomenon of NMR is the perturbation of the nuclear moments, which are in a field H_0, by a small alternating field with a strength H_1, perpendicular to the H_0. Resonance occurs at the coincidence of field rotation speeds H_1 and ω_0 nuclei in the field H_0.

10.9 Chemical Shift

The effective magnetic field at which there is resonance absorption of the proton depends on the strength of the magnetic fields produced by the interaction of the applied magnetic field H_0 with the electronic system of the molecule. The magnitude of these magnetic fields (N_{el}) is proportional to the applied magnetic field:

$$H_{el} = -\sigma H_0$$

where σ is called the screening constant.

The effective field, at which resonance is carried out, will be:

$$H_{ef} = H_0 - \sigma H_0 = H_0(1-\sigma)$$

Since protons in the molecule are in different environments, they are differently shielded. Resonance conditions for them are different and in the spectrum they correspond to separate signals. Signals of equally shielded nuclei are coincided, and such nuclei are called chemically equivalent. Consequently, the number of signals which appear in the spectrum is determined by the number of different shielded protons.

The distance between the signals of two different shielded protons A and X is called the chemical shift. The chemical shift, measured in Hz, is proportional to the applied field.

Practical chemical shift is defined relative to the signal of the reference material and is measured in parts per million.

As a standard it is selected tetramethylsilane $(CH3)_4Si$. Its protons signal is in the stronger field than the signals of protons of the majority organic compounds. Tetramethylsilane is chemically inert, magnetically isotropic, volatile liquid that easily miscible with most of the solvents. Its signal is taken as 0 and the value t (ppm) increases in the direction of the weak fields.

10.10 Factors Influencing on the Chemical Shift

The electron density in the proton effects on the magnitude of the chemical shift. In the fragment Y-X-H it depends on electronegativity of the neighboring atom X and the inductive effect of neighboring groups Y. The circulation of the electrons connection X-H in the field H_0 induces a local magnetic field, which is in the location of the proton that always directed against H_0. Thus, the resulting magnetic field shields the proton from the field H_0, and the larger the screening, in the stronger fields is observed signal of the proton.

On the chemical shift of protons also influences the magnetic field resulting from electrons circulation in other parts of the molecule. These fields can have on the proton both screening and unscreening effect, which can be illustrated by the example of benzene. If the benzene molecule is oriented perpendicular to the direction of the field H_0 then circulation of the p-electrons induces a field. The protons of aromatic ring are in the area where the direction of two fields matches. Therefore occurs the unscreening of the proton, and its signal is observed in a weak field (7-8 ppm). It should be remembered that as a result of Brownian motion, the orientation of benzene molecule with respect to the magnetic field H_0 may be different, but on the chemical shift impact only specifically oriented molecules.

Screening and unscreening fields for magnetically anisotropic groups are usually depicted by so-called cone anisotropy. In Fig. 12 are represented cones of anisotropy for some groups. Plus sign specifies the area of screening, minus - unscreening. The signals of protons that entering the area of screening are observed in strong fields (such as acetylene proton has s = 2.35 ppm), and in the unscreening area are manifested in weak fields (the protons of the aldehyde group have s = 9-10 m. etc.). Anisotropic effects possess more or less all the groups and links. The anisotropy of a single bond leads to a difference in the situation of the signals of axial and equatorial protons in cyclohexane hard systems.

Thus, position of the resonance signal (chemical shift) is determined by the totality of the electron density around the considered proton and anisotropic effects of neighboring groups.

It should be noted that the position of the hydroxyl proton signals and the amino group can be varied within wide limits. This is due to their ability to form hydrogen bonds, which leads changing in the proton

screening. If in the solution there is a balance between free and bound molecules of hydrogen bonds, then position of the signal is determined by the equilibrium state and depends on the concentration, temperature and solvent. The signal appears in the region that is intermediate between the signals of the free and fully connected X-H group. It is useful to recall that in the IR spectra of free and bound hydrogen-bonded hydroxyl and amino groups are recorded separately and the equilibrium state is determined by the intensity of their absorption bands.

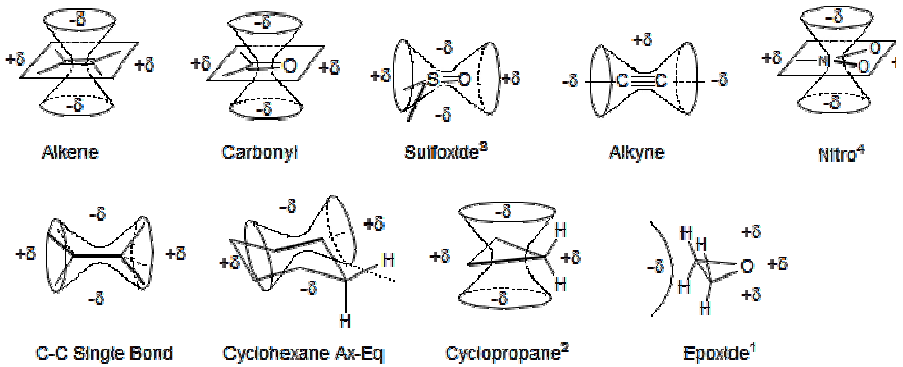


Fig. 12 Anisotropy cones for some groups.

10.11 Preparation of Samples and Standards for NMR Spectroscopy

In most cases, the NMR spectra recorded for non-viscous liquids and solutions. Thus sample preparation provides for the choice of ampoule, solvent, solution concentration, the standard for measuring of chemical shifts and, if necessary, shifting reagents, calibration standards and other additives. Liquid or solution should be thoroughly cleaned and filtered from any heterogeneous particles. Especially important to remove the paramagnetic and ferromagnetic impurities, since they lead to an extremely strong line broadening and even to the spectrum disappearance. At the same time, as was noted above, the addition of some paramagnetic complexes that is shifting reagent does not spoil the spectrum, but it is even useful. It is also important to monitor temperature of the sample.

In NMR spectroscopy are used standard solvents such as carbon tetrachloride, chloroform and deuterium rohloroform, acetone and deuterium acetone, benzene and benzene deuterium, dimethyl sulfoxide, etc. Various special solvents are used at high and low temperatures (e.g. deuterium-dimethyl sulfoxide, and methanol or Freon-12), as well as in the case of selective dissolution of the samples (e.g. formic acid and deuterated water).

Optimal concentrations are chosen within the allowable level of the signal / noise ratio and the solubility of the substance. In the PMR spectroscopy the content of substances in solution is usually expressed in molar fractions (%), which is useful in assessing the relative intensities of the signals.

If we talk about polymers, they are before taking the NMR spectra are previously dissolved in deuterated solvents. The values of chemical shifts in polymers follow the same rules that apply to organic compounds of low molecular weight. Often, however, the signals of the protons or other nuclei of polymers isotopes are less clear, and "fuzzy" due to the presence of molecular weight distribution.

The standards used to measure the chemical shifts are different. In practice are used the methods of internal or external standard. In the first method, the standard (e.g. 1-2 drops of tetramethylsilane (or TMS)) is injected directly into the solution of the substance. It is assumed that the standard inert with respect to the solvent and solute (does not form associates, etc.). When it is not, it is used a method of external standard. In this case the solution of the standard (e.g. TMS) is filled to an ampoule of smaller diameter, placed it in the main ampoule and somehow centered in it. In the second method is necessary to introduce a correction on differences in bulk diamagnetic susceptibility of solutions of the test substance and standard.

In NMR spectroscopy depends on different nuclei there are own methodological features of the sample preparation. For example, for ^{13}C NMR is usually required a larger diameter of ampoule (8-25 mm) than for TMR (~ 5 mm), and as standards in the chemical shifts, in addition to TMS, sometimes are used deuterated compounds.

10.12 NMR Application in Polymer Branch

In addition to establishing the chemical structure of polymers, NMR spectroscopy is used to determine:

- Molecular mobility;
- The kinetics of polymerization processes;
- Determining the proportion of crystalline and amorphous phases;
- Self-diffusion coefficient.

11. ELECTRON PARAMAGNETIC RESONANCE

Electron Paramagnetic Resonance (EPR) or **Electron Spin Resonance** (ESR) spectroscopy is a technique for studying chemical species that have one or more unpaired electrons, such as organic and inorganic free radicals or inorganic complexes possessing a transition metal ion. The basic physical concepts of EPR are analogous to those of nuclear magnetic resonance (NMR), but it is electron spins that are excited instead of spins of atomic nuclei. Because most stable molecules have all their electrons paired, the EPR technique is less widely used than NMR. However, this limitation to paramagnetic species also means that the EPR technique is one of great specificity, since ordinary chemical solvents and matrices do not give rise to EPR spectra.

EPR was first observed in Kazan State University by Soviet physicist Yevgeny Zavoisky in 1944, and was developed independently at the same time by Brebis Bleaney at the University of Oxford.

EPR is the phenomenon of resonant absorption of electromagnetic waves energy by paramagnetic particles placed in a constant magnetic field.

Energy absorption induces transitions between energy levels due to the different orientation of the magnetic moments of electrons (and not the nuclei, as in the case of NMR) in the space. Since the magnetic and mechanical moment of completely filled electron shells of atoms is zero, the EPR method is only applicable for the systems with nonzero total spin angular momentum of electrons, i.e. for paramagnetic systems with an incomplete shell. These are:

1. free radicals in solid, liquid and gaseous phases (a molecule containing an unpaired electron);

2. some point defects (local destruction of the crystal lattice) in solids. This group was studied centers of electrons captured by negative ion vacancies; lack of an electron (the "positive hole") may also form a paramagnetic center;

3. biradicals are molecules that contain two unpaired electrons, removed to a distance whree interaction between them is very weak; such molecules behave as two weakly interacting free radicals;

4. systems in the triplet state (two unpaired electrons). There are molecules for which the triplet state is a major, and the molecules passing into the triplet state by thermal or optical excitation;

5. systems with three or more unpaired electrons;

6. ions of transition and rare earth elements.

EPR is caused by the orientation of the unpaired electrons in a magnetic field so that their intrinsic angular momentum (spin) is directed along the field, or against it. The energy difference between these two states is called the energy of the Zeeman splitting, it is equal to $h\nu=g\mu_B H$ where g − the spectroscopic splitting factor, H − magnetic field strength, μ_B − the magnetic moment (Bohr magneton).

An alternating electromagnetic field with the energy of the Zeeman splitting is applied in a direction perpendicular to the static magnetic field and induced a reorientation of the electrons, i.e. transition between the Zeeman levels. As the number of electrons at a lower level is higher than at the top then the number of upwards transitions with the absorption of energy will prevail over the number of downwards transition. As a result, occurs the energy absorbtion of high-frequency field and appears a signal of electron spin resonance.

11.1 Origin of an EPR Signal

Every electron has a magnetic moment and spin quantum number s = 1/2, with magnetic components $m_s = +1/2$ and $m_s = -1/2$. In the presence of an external magnetic field with strength B_0, the electron's magnetic moment aligns itself either parallel ($m_s = -1/2$) or antiparallel ($m_s = +1/2$) to the field, each alignment having a specific energy (see the Zeeman effect). The parallel alignment corresponds to the lower energy state, and the separation between it and the upper state is $\Delta E = g_e \mu_B B_0$, where g_e is the electron's so-called g-factor and μ_B is the Bohr magneton. This equation implies that the splitting of the energy levels is directly proportional to the magnetic field's strength, as shown in the diagram below.

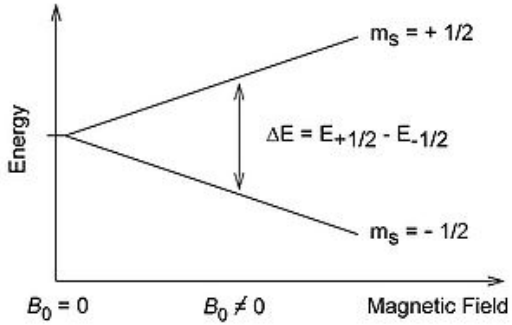


Fig. 13 Splitting of the energy levels.

An unpaired electron can move between the two energy levels by either absorbing or emitting electromagnetic radiation of energy $\epsilon = h\nu$ such that the resonance condition, $\epsilon = \Delta E$, is obeyed. Substituting in $\epsilon = h\nu$ and $\Delta E = g_e\mu_B B_0$ leads to the fundamental equation of EPR spectroscopy: $h\nu = g_e\mu_B B_0$. Experimentally, this equation permits a large combination of frequency and magnetic field values, but the great majority of EPR measurements are made with microwaves in the 9000–10000 MHz (9–10 GHz) region, with fields corresponding to about 3500 G (0.35 T). See below for other field-frequency combinations.

In principle, EPR spectra can be generated by either varying the photon frequency incident on a sample while holding the magnetic field constant or doing the reverse. In practice, it is usually the frequency that is kept fixed. A collection of paramagnetic centers, such as free radicals, is exposed to microwaves at a fixed frequency. By increasing an external magnetic field, the gap between the $m_s = +1/2$ and $m_s = -1/2$ energy states is widened until it matches the energy of the microwaves, as represented by the double-arrow in the diagram below. At this point the unpaired electrons can move between their two spin states. Since there typically are more electrons in the lower state, due to the Maxwell-Boltzmann distribution (see Fig. 14), there is a net absorption of energy, and it is this absorption that is monitored and converted into a spectrum.

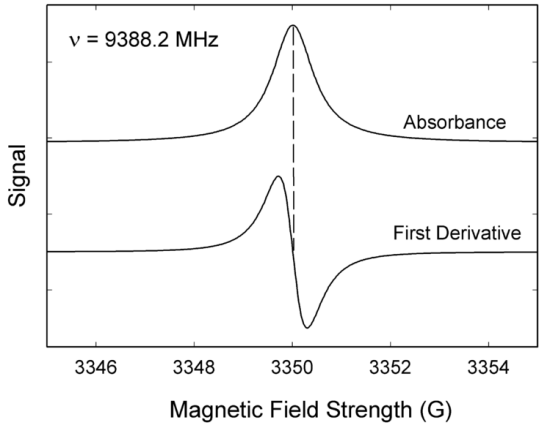


Fig. 14 EPR spectra.

As an example of how $h\nu = g_e\mu_B B_0$ can be used, consider the case of a free electron, which has $g_e = 2.0023$, and the simulated spectrum shown at the right in two different forms. For the microwave frequency of

9388.2 MHz, the predicted resonance position is a magnetic field of about $B_0 = h\nu/ g_e\mu_B = 0.3350$ tesla = 3350 gauss, as shown. Note that while two forms of the same spectrum are presented in the figure, most EPR spectra are recorded and published only as first derivatives.

Because of electron-nuclear mass differences, the magnetic moment of an electron is substantially larger than the corresponding quantity for any nucleus, so that a much higher electromagnetic frequency is needed to bring about a spin resonance with an electron than with a nucleus, at identical magnetic field strengths. For example, for the field of 3350 G shown at the right, spin resonance occurs near 9388.2 MHz for an electron compared to only about 14.3 MHz for ^{1}H nuclei. (For NMR spectroscopy, the corresponding resonance equation is $h\nu = g_N\mu_N B_0$ where g_N and μ_N depend on the nucleus under study).

11.2 EPR Method Design

EPR spectrometer is a device used for the detection of magnetic dipole transitions. Monochromatic electromagnetic radiation is supplied to the sample and the detector is observed for changes in the intensity of the radiation transmitted through the sample. By changing the static magnetic field is found its resonant value $H = h\nu/g\mu\nu$ at which the absorption signal is detected. Typically, the EPR spectrum is observed at a constant frequency ac field (9,000 MHz) and recorded the first derivative of this signal.

Alternating high-frequency field applied perpendicular to the direction of the static magnetic field causes the reorientation of the unpaired electrons, i.e. induces electron transitions between the Zeeman levels. This field "throws" the electrons to the upper level and "resets" them from the upper to the lower level, with equal probability. But as the number of electrons at a lower level is higher than at the top then the number of upwards transitions is more than in the opposite direction. At high power of high-frequency field population of both Zeeman levels are aligned, the energy absorption of high-frequency field is absent, and the EPR signal is disappeared − this is the saturation of the EPR.

Saturation is removed, when the electrons move spontaneously from the upper to the lower level, thus supporting the excess population of the lower level. This process is induced by the interaction of an electron with its surroundings (lattice) and is called the spin-lattice relaxation characterized by relaxation time T2. Radicals with short relaxation times are saturated at high power of high-frequency field; radicals with long re-

laxation times are saturated easily at low power field. By study the dependence of the EPR spectra on high-power field, i.e. saturate successively radicals with different relaxation times, it is possible to divide EPR spectra of different radicals. This method is called the differential saturation.

A typical continuous wave (CW) EPR spectrometer (see Fig. 15) consists of a source of microwave radiation, a cavity into which the sample is placed (to enhance the size of the microwave field), a detector to measure the microwave signal reflected from the cavity and a magnet to generate the external field. A small modulation field allows the use of phase sensitive detection.

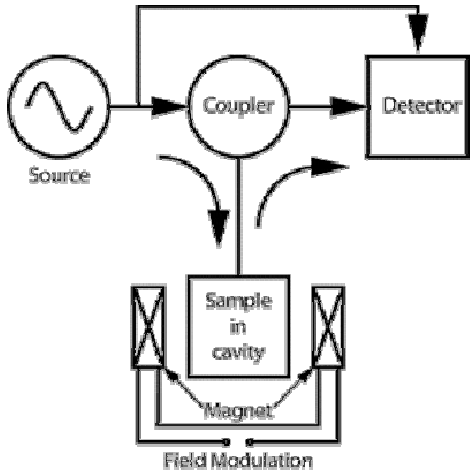


Fig. 15 EPR device scheem.

11.3 EPR Methods

Ferromagnetic Resonance, or FMR, is a spectroscopic technique to probe the magnetization of ferromagnetic materials. It is a standard tool for probing spin waves and spin dynamics. FMR is very broadly similar to electron paramagnetic resonance (EPR), and also somewhat similar to nuclear magnetic resonance (NMR) except that FMR probes the sample magnetization resulting from the magnetic moments of dipolar-coupled but unpaired electrons whereas NMR probes the magnetic moment of atomic nuclei screened by the atomic or molecular orbitals surrounding such nuclei of non-zero nuclear spin.

Site-Directed Spin Labeling (SDSL) is a technique for investigating protein local dynamics using electron spin resonance. The theory of SDSL is based on the specific reaction of spin labels with amino acids. A spin label's built-in protein structure can be detected by EPR spectroscopy. SDSL is also a useful tool in examinations of protein folding process.

A Spin Label (**SL**) is an organic molecule which possesses an unpaired electron, usually on a nitrogen atom, and the ability to bind to another molecule. Spin labels are normally used as tools for probing proteins or biological membrane-local dynamics using EPR spectroscopy. The site-directed spin labeling (SDSL) technique allows one to monitor a specific region within a protein. In protein structure examinations, amino acid-specific SLs can be used.

Spin Trapping in chemistry is an analytical technique employed in the detection and identification of short-lived free radicals. Spin trapping involves the addition of radical to a nitrone spin trap resulting in the formation of a spin adduct, a nitroxide-based persistent radical, that can be detected using electron paramagnetic resonance (EPR) spectroscopy. The spin adduct usually yields a distinctive EPR spectrum characteristic of a particular free radical that is trapped. The identity of the radical can be inferred based from the EPR spectral profile of their respective spin adducts such as the *g* value, but most importantly, the hyperfine-coupling constants of relevant nuclei. Unambiguous assignments of the identity of the trapped radical can often be made by using stable isotope substitution of the radicals' parent compound, so that further hyperfine couplings are introduced or altered.

12. THERMAL ANALYSIS OF POLYMERS

Polymers represent another large area in which thermal analysis finds strong applications. Thermoplastic polymers are commonly found in everyday packaging and household items, but for the analysis of the raw materials, effects of the many additive used (including stabilisers and colours) and fine-tuning of the moulding or extrusion processing used can be achieved by using DSC. An example is oxidation induction time (OIT) by DSC which can determine the amount of oxidation stabiliser present in a thermoplastic (usually a polyolefin) polymer material. Compositional analysis is often made using TGA, which can separate fillers, polymer resin and other additives. TGA can also give an indication of thermal stability and the effects of additives such as flame retardants.

Thermal analysis of composite materials, such as carbon fibre composites or glass epoxy composites are often carried out using DMA or DMTA, which can measure the stiffness of materials by determining the modulus and damping (energy absorbing) properties of the material. Aerospace companies often employ these analysers in routine quality control to ensure that products being manufactured meet the required strength specifications. Formula 1 racing car manufacturers also have similar requirements! DSC is used to determine the curing properties of the resins used in composite materials, and can also confirm whether a resin can be cured and how much heat is evolved during that process. Application of predictive kinetics analysis can help to fine-tune manufacturing processes. Another example is that TGA can be used to measure the fibre content of composites by heating a sample to remove the resin by application of heat and then determining the mass remaining.

12.1 Differential Thermal Analysis

Differential Thermal Analysis (or DTA) is a thermoanalytic technique, similar to differential scanning calorimetry. In DTA, the material under study and an inert reference are made to undergo identical thermal cycles, while recording any temperature difference between sample and reference. This differential temperature is then plotted against time, or against temperature (DTA curve or thermogram). Changes in the sample, either exothermic or endothermic, can be detected relative to the inert reference. Thus, a DTA curve provides data on the transformations that have occurred, such as glass transitions, crystallization, melting and sublimation. The area under a DTA peak is the enthalpy change and is not affected by the heat capacity of the sample.

In the thermogravimetric analysis the recorded material characteristic is temperature T as a function of time t. At the same time thermogram is recorded in the coordinates of T-t. The most valuable information is obtained by differential thermal analysis (DTA) in which is measured temperature difference Δ T of the sample and an inert reference. As a standard is used a substance, that does not undergo thermal decomposition in the applied temperature range. Thus thermogram is recorded in the coordinates Δ T - t.

DTA is used to study both the chemical and physical transformations in polymers.

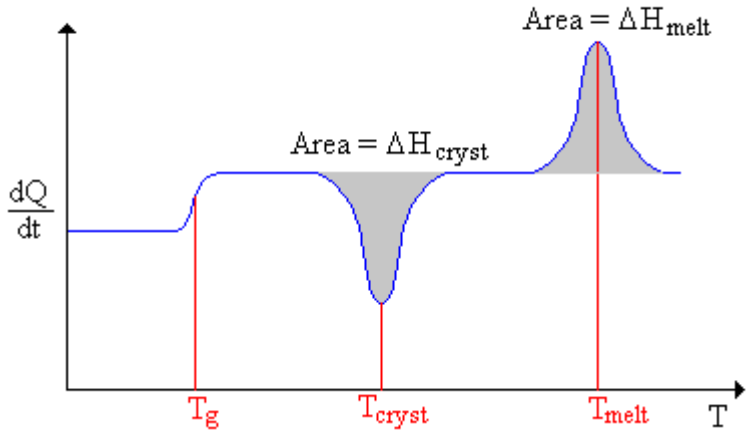


Fig. 16 Physical transitions in polymers.

Physical transitions in polymers studied by DTA are ordered as follows: glass transition, a "cold" crystallization, crystal-crystal transitions, crystallization from the melt, melting.

12.2 Calorimetry

Calorimetry is the science of measuring the heat of chemical reactions or physical changes. Calorimetry is performed with a calorimeter. The word calorimetry is derived from the Latin word calor, meaning heat. Scottish physician and scientist Joseph Black, who was the first to recognize the distinction between heat and temperature, is said to be the founder of calorimetry.

Calorimetry is the measurement of the released or absorbed heat quantity by the sample in the process of heating. The main method of calorimetric analysis is differential scanning calorimetry (DSC). DSC method is very suitable for the analysis of phase transitions in materials. DSC allows quickly and easily determines the melting and crystallization temperature of the substance as well as temperature of solid-phase and phase transitions in liquid crystals. The results of these studies take the form of exothermic or endothermic peaks in DSC curves and thermograms.

Differential scanning calorimetry or DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of tem-

perature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

The main properties of the material studied by DSC:

- The behavior during melting;
- Glass transition;
- Crystallization;
- Oxidation resistance;
- Kinetics;
- Purity degree;
- Specific heat.

The basic principle underlying this technique is that when the sample undergoes a physical transformation such as phase transitions, more or less heat will need to flow to it than the reference to maintain both at the same temperature. Whether less or more heat must flow to the sample depends on whether the process is exothermic or endothermic. For example, as a solid sample melts to a liquid it will require more heat flowing to the sample to increase its temperature at the same rate as the reference. This is due to the absorption of heat by the sample as it undergoes the endothermic phase transition from solid to liquid. Likewise, as the sample undergoes exothermic processes (such as crystallization) less heat is required to raise the sample temperature. By observing the difference in heat flow between the sample and reference, differential scanning calorimeters are able to measure the amount of heat absorbed or released during such transitions. DSC may also be used to observe more subtle phase changes, such as glass transitions. It is widely used in industrial settings as a quality control instrument due to its applicability in evaluating sample purity and for studying polymer curing.

Applications

Polymers. DSC is used widely for examining polymers to check their composition. Melting points and glass transition temperatures for most polymers are available from standard compilations, and the method can show possible polymer degradation by the lowering of the expected melting point, T_m, for example. T_m depends on the molecular weight of the polymer, so lower grades will have lower melting points than expected. The percentage crystallinity of a polymer can be found from the crystallization peak of the DSC graph since the heat of fusion can be calculated from the area under an absorption peak. DSC can also be used to study thermal degradation of polymers. Impurities in polymers can be determined by examining thermograms for anomalous peaks, and plasticisers can be detected at their characteristic boiling points.

Liquid Crystals. DSC is used in the study of liquid crystals. As some forms of matter go from solid to liquid they go through a third state, which displays properties of both phases. This anisotropic liquid is known as a liquid crystalline or mesomorphous state. Using DSC, it is possible to observe the small energy changes that occur as matter transitions from a solid to a liquid crystal and from a liquid crystal to an isotropic liquid.

Oxidative Stability. Using differential scanning calorimetry to study the stability to oxidation of samples generally requires an airtight sample chamber. Usually, such tests are done isothermally (at constant temperature) by changing the atmosphere of the sample. First, the sample is brought to the desired test temperature under an inert atmosphere, usually nitrogen. Then, oxygen is added to the system. Any oxidation that occurs is observed as a deviation in the baseline. Such analysis can be used to determine the stability and optimum storage conditions for a material or compound.

12.3 Thermogravimetry

Thermogravimetric Analysis or **Thermal Gravimetric Analysis** (TGA) is a type of testing performed on samples that determines changes in weight in relation to change in temperature. Such analysis relies on a high degree of precision in three measurements: weight, temperature, and temperature change. As many weight loss curves look similar, the weight loss curve may require transformation before results may be interpreted. A derivative weight loss curve can identify the point where weight loss is most apparent. Again, interpretation is limited without further modifications and deconvolution of the overlapping peaks may be required. To determine composition and purity one must take the mass of the substance in the mixture by using thermal gravimetric analysis. Thermal gravimetric analysis is the act of heating a mixture to a high enough temperature so that one of the components decomposes into a gas, which dissociates into the air. It is a process that utilizes heat and stoichiometry ratios to determine the percent by mass ration of a solute. If the compounds in the mixture that re-

main are known, then the percentage by mass can be determined by taking the weight of what is left in the mixture and dividing it by the initial mass. Knowing the mass of the original mixture and the total mass of impurities liberating upon heating, the stoichiometric ratio can be used to calculate the percent mass of the substance in a sample. TGA is commonly employed in research and testing to determine characteristics of materials such as polymers, to determine degradation temperatures, absorbed moisture content of materials, the level of inorganic and organic components in materials, decomposition points of explosives, and solvent residues. It is also often used to estimate the corrosion kinetics in high temperature oxidation.

Simultaneous TGA-DTA/DSC measures both heat flow and weight changes (TGA) in a material as a function of temperature or time in a controlled atmosphere. Simultaneous measurement of these two material properties not only improves productivity but also simplifies interpretation of the results. The complementary information obtained allows differentiation between endothermic and exothermic events with no associated weight loss (e.g. melting and crystallization) and those that involve a weight loss (e.g. degradation).

The analyzer usually consists of a high-precision balance with a pan (generally platinum) loaded with the sample. A different process using a quartz crystal microbalance has been devised for measuring smaller samples on the order of a microgram (versus milligram with conventional TGA). The sample is placed in a small electrically heated oven with a thermocouple to accurately measure the temperature. The atmosphere may be purged with an inert gas to prevent oxidation or other undesired reactions. A computer is used to control the instrument.

TGA is a process that utilizes heat and stoichiometry ratios to determine the percent by mass of a solute. Analysis is carried out by raising the temperature of the sample gradually and plotting weight (percentage) against temperature. The temperature in many testing methods routinely reaches 1000°C or greater. After the data are obtained, curve smoothing and other operations may be done to find the exact points of inflection.

A method known as hi-resolution TGA is often employed to obtain greater accuracy in areas where the derivative curve peaks. In this method, temperature increase slows as weight loss increases. This is to more accurately identify the exact temperature where a peak occurs. Several modern TGA devices can vent burnoff to an infrared spectrophotometer to analyze composition.

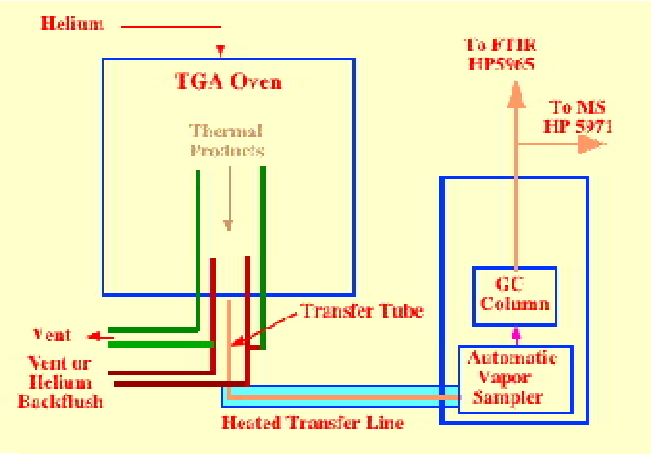


Fig. 17 TGA apparatus diagram.

12.4 Dilatometry

Dilatometry is also used to monitor the progress of chemical reactions, particularly those displaying a substantial molar volume change (e.g., polymerization). A specific example is the rate of phase changes.

A dilatometer is a scientific instrument that measures volume changes caused by a physical or chemical process. A familiar application of a dilatometer is the mercury-in-glass thermometer, in which the change in volume of the liquid column is read from a graduated scale. Because mercury has a fairly constant rate of expansion over normal temperature ranges, the volume changes are directly related to temperature.

Application areas:

- Determination of the polymers thermal expansion coefficient;
- Determination of glass transition temperature;
- Determination of softening point;
- Determination of the temperature and kinetics of melting and crystallization.

There are a number of dilatometer types:

- Capacity dilatometers possess a parallel plate capacitor with a one stationary plate, and one moveable plate. When the sample length changes, it moves the moveable plate, which changes the gap between the plates. The capacitance is inversely proportional to the gap. Changes in length of 10 picometres can be detected.

• Connecting rod (push rod) dilatometer, the sample which can be examined is in the furnace. A connecting rod transfers the thermal expansion to a strain gauge, which measures the shift. Since the measuring system (connecting rod) is exposed to the same temperature as the sample and thereby likewise expands, one obtains a relative value, which must be converted afterwards. Matched low-expansion materials and differential constructions can be used to minimize the influence of connecting rod expansion.

• High Resolution - Laser Dilatometer Highest resolution and absolute accuracy is possible with a Michelson Interferometer type Laser Dilatometer. Resolution goes up to picometres. On top the principle of interference measurement give the possibility for much higher accuracy's and it is an absolute measurement technique with no need of calibration.

• Optical dilatometer is an instrument that measures dimension variations of a specimen heated at temperatures that generally range from 25 to 1400°C. The optical dilatometer allows the monitoring of materials' expansions and contractions by using a non-contact method: optical group connected to a digital camera captures the images of the expanding/contracting specimen as function of the temperature with a resolution of about ±70 micrometre per pixel. As the system allows to heat up the material and measures its longitudinal/vertical movements without any contact between instrument and specimen, it is possible to analyse the most ductile materials, such as the polymers, as well as the most fragile, such as the incoherent ceramic powders for sintering process.

For simpler measurements in a temperature range from 0 to 100°C, where water is heated up and flow or over the sample. If linear coefficients of expansion of a metal is to be measured, hot water will running through a pipe made from the metal. The pipe warms up to the temperature of the water and the relative expansion can be determined as a function of the water temperature.

For the measurement of the volumetric expansion of liquids one takes a large glass container filled with water. In an expansion tank (glass container with an accurate volume scale) with the sample liquid. If one heats the water up, the sample liquid expands and the volume changes is read. However the expansion of the sample container must also be taken into consideration.

The expansion and retraction coefficient of gases cannot be measured using dilatometer, since the pressure plays a role here. For such measurements a gas thermometer is more suitable.

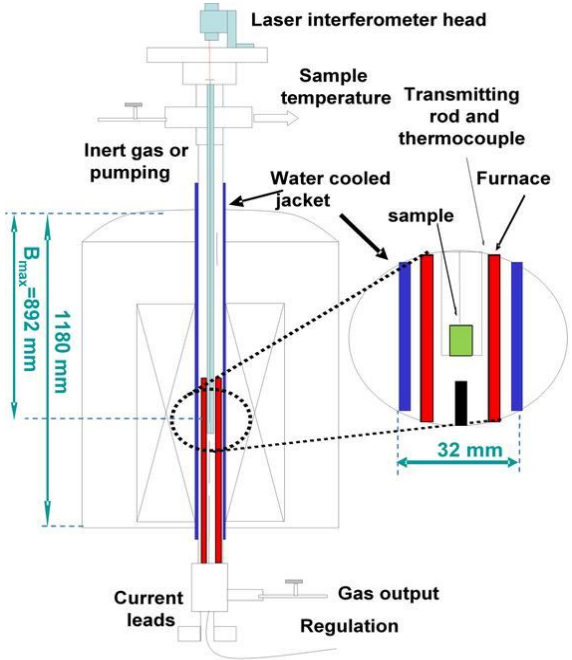


Fig. 18 Dilatometer device scheme.

Dilatometers often include a mechanism for controlling temperature. This may be a furnace for measurements at elevated temperatures (temperatures to 2000°C), or a cryostat for measurements at temperatures below room temperature. Metallurgical applications often involve sophisticated temperature controls capable of applying precise temperature-time profiles for heating and quenching the sample.

12.5 Thermomechanics

Thermomechanical Analysis (TMA) is a technique used in thermal analysis, a branch of materials science which studies the properties of materials as they change with temperature.

Thermomechanical analysis is a subdiscipline of the themomechanometry (TM) technique.

Thermomechanometry is the measurement of a change of a dimension or a mechanical property of the sample while it is subjected to a temperature regime. An associated thermoanalytical method is thermomechanical analysis. A special related technique is thermodilatometry (TD), the measurement of a change of a dimension of the sample with a negligible force acting on the sample while it is subjected to a temperature regime. The associated thermoanalytical method is thermodilatometric analysis (TDA).

TDA is often referred to as zero force TMA. The temperature regime may be heating, cooling at a rate of temperature change that can include stepwise temperature changes, linear rate of change, temperature modulation with a set frequency and amplitude, free (uncontrolled) heating or cooling, or maintaining a constant increase in temperature. The sequence of temperatures with respect to time may be predetermined (temperature programmed) or sample controlled (controlled by a feedback signal from the sample response).

Thermomechanometry includes several variations according to the force and the way the force is applied.

Static force TM (sf-TM) is when the applied force is constant; previously called TMA with TD as the special case of zero force.

Dynamic force TM (df-TM) is when the force is changed as for the case of a typical stress–strain analysis; previously called TMA with the term dynamic meaning any alteration of the variable with time, and not to be confused with dynamic mechanical analysis (DMA).

Modulated force TM (mf-TM) is when the force is changed with a frequency and amplitude; previously called DMA. The term modulated is a special variant of dynamic, used to be consistent with modulated temperature differential scanning calorimetry (mt-DSC) and other situations when a variable is imposed in a cyclic manner.

Mechanical Test

Mechanical testing seeks to measure mechanical properties of materials using various test specimen and fixture geometries using a range of probe types.

Measurement is desired to take place with minimal disturbance of the material being measured. Some characteristics of a material can be measured without disturbance, such as dimensions, mass,volume, density.

However, measurement of mechanical properties normally involves disturbance of the system being measured.

The measurement often reflects the combined material and measuring device as the system. Knowledge of a structure can be gained by imposing an external stimulus and measuring the response of the material with a suitable probe. The external stimulus can be a stress or strain, however in thermal analysis the influence is often temperature.

Thermomechanometry is where a stress is applied to a material and the resulting strain is measured while the material is subjected to a controlled temperature program. The simplest mode of TM is where the imposed stress is zero. No mechanical stimulus is imposed upon the material; the material response is generated by a thermal stress, either by heating or cooling.

Zero Force Thermomechanometry

Zero force TM (a variant of sf-TM or TD) measures the response of the material to changes in temperature and the basic change is due to activation of atomic or molecular phonons. Increased thermal vibrations produce thermal expansion characterized by the coefficient of thermal expansion (CTE) that is the gradient of the graph of dimensional change versus temperature.

CTE depends upon thermal transitions such as the glass transition. CTE of the glassy state is low, while at the glass transition temperature (Tg) increased degrees of molecular segmental motion are released so CTE of the rubbery state is high. Changes in an amorphous polymer may involve other sub-Tg thermal transitions associated with short molecular segments, side-chains and branches. The linearity of the sf-TM curve will be changed by such transitions.

Other relaxations may be due to release of internal stress arising from the non-equilibrium state of the glassy amorphous polymer. Such stress is referred to as thermal aging. Other stresses may be as a result of moulding pressures, extrusion orientation, thermal gradients during solidification and externally imparted stresses.

Semi-Crystalline Polymers

Semi-crystalline polymers are more complex than amorphous polymers, since the crystalline regions are interspersed with amorphous regions. Amorphous regions in close association to the crystals or contain

common molecules as tie molecules have less degrees of freedom than the bulk amorphous phase. These immobilised amorphous regions are called the rigid amorphous phase. CTE of the rigid amorphous phase is expected to be lower than that of the bulk amorphous phase.

The crystallite are typically not at equilibrium and they may contain different polymorphs. The crystals re-organize during heating so that they approach the equilibrium crystalline state. Crystal re-organization is a thermally activated process. Further crystallization of the amorphous phase may take place. Each of these processes with interfere with thermal expansion of the material.

The material may be a blend or a two-phase block or graft copolymer. If both phases are amorphous then two Tg will be observed if the material exists as two phases. If one Tg is exhibited then it will be between the Tg of the components and the resultant Tg will likely be described by a relationship such as the Flory-Fox or Kwei equations.

If one of the components is semi-crystalline then the complexity of a pure crystalline phase and either one or two amorphous phases will result. If both components are semi-crystalline then themorphology will be complex since both crystal phases will likely form separately, though with influence on each other.

Cross-Linking

Cross-linking will restrict the molecular response to temperature change since degree of freedom for segmental motions are reduced as molecules become irreversibly linked. Crosslinking chemically links molecules, while crystallinity and fillers introduce physical constraints to motion. Mechanical properties such as derived from stress-strain testing are used to calculate crosslink density that is usually expressed as the molar mass between crosslinks (Mc).

The sensitivity of zero stress TMA to crosslinking is low since the structure receives minimum disturbance. Sensitivity to crosslinks requires high strain such that the segments between crosslinks become fully extended.

Zero force TM will only be sensitive to changes in the bulk that are expressed as a change in a linear dimension of the material. The measured change will be the resultant of all processes occurring as the temperature is changed. Some of the processes with be reversible, others irreversible, and

others time dependent. The methodology must be chosen to best detect, distinguish and resolve the thermal expansion or contractions observable.

The TM instrument need only apply sufficient stress to keep the probe in contact with the specimen surface, but it must have high sensitivity to dimensional change. The experiment must be conducted at a temperature change rate slow enough for the material to approach thermal equilibrium throughout. While the temperature should be the same throughout the material it will not necessarily be at thermal equilibrium in the context of molecular relaxations.

The temperature of the molecules relative to equilibrium is expressed as the fictive temperature. The fictive temperature is the temperature at which the unrelaxed molecules would be at equilibrium.

Application Areas:

- softening temperature;
- glass transition temperature;
- deformability of polymers;
- elastic modulus;
- angle tangent of mechanical losses;
- phase transitions.

12.6 Glass Transition Temperature

The **Glass-Liquid Transition** (or **Glass Transition** for short) is the reversible transition in amorphous materials (or in amorphous regions within semicrystalline materials) from a hard and relatively brittle state into a molten or rubber-like state. An amorphous solid that exhibits a glass transition is called a glass. Supercooling a viscous liquid into the glass state is called vitrification, from the Latin vitreum, "glass" via French vitrifier.

Despite the massive change in the physical properties of a material through its glass transition, the transition is not itself a phase transition of any kind; rather it is a laboratory phenomenon extending over a range of temperature and defined by one of several conventions. Such conventions include a constant cooling rate (20 K/min) and a viscosity threshold of 10^{12} Pa·s, among others. Upon cooling or heating through this glass transition range, the material also exhibits a smooth step in the thermal expansion coefficient and in the specific heat, with the location of these effects again being dependent on the history of the material. However, the question of

whether some phase transition underlies the glass transition is a matter of continuing research.

The glass transition temperature T_g is always lower than the melting temperature, T_m, of the crystalline state of the material, if one exists.

The glass transition of a liquid to a solid-like state may occur with either cooling or compression. The transition comprises a smooth increase in the viscosity of a material by as much as 17 orders of magnitude without any pronounced change in material structure. The consequence of this dramatic increase is a glass exhibiting solid-like mechanical properties on the timescale of practical observation. This transition is in contrast to the freezing or crystallization transition, which is a first-order phase transition in the Ehrenfest classification and involves discontinuities in thermodynamic and dynamic properties such as volume, energy, and viscosity. In many materials that normally undergo a freezing transition, rapid cooling will avoid this phase transition and instead result in a glass transition at some lower temperature. Other materials, such as many polymers, lack a well defined crystalline state and easily form glasses, even upon very slow cooling or compression.

Below the transition temperature range, the glassy structure does not relax in accordance with the cooling rate used. The expansion coefficient for the glassy state is roughly equivalent to that of the crystalline solid. If slower cooling rates are used, the increased time for structural relaxation (or intermolecular rearrangement) to occur may result in a higher density glass product. Similarly, byannealing (and thus allowing for slow structural relaxation) the glass structure in time approaches an equilibrium density corresponding to the supercooled liquid at this same temperature. T_g is located at the intersection between the cooling curve (volume versus temperature) for the glassy state and the supercooled liquid.

The configuration of the glass in this temperature range changes slowly with time towards the equilibrium structure. The principle of the minimization of the Gibbs free energy provides the thermodynamic driving force necessary for the eventual change. It should be noted here that at somewhat higher temperatures than T_g, the structure corresponding to equilibrium at any temperature is achieved quite rapidly. In contrast, at considerably lower temperatures, the configuration of the glass remains sensibly stable over increasingly extended periods of time.

Thus, the liquid-glass transition is not a transition between states of thermodynamic equilibrium. It is widely believed that the true equilibrium state is always crystalline. Glass is believed to exist in a kinetically locked state, and its entropy, density, and so on, depends on the thermal history. Therefore, the glass transition is primarily a dynamic phenomenon. Time and temperature are interchangeable quantities (to some extent) when dealing with glasses, a fact often expressed in the time-temperature superposition principle. On cooling a liquid, internal degrees of freedom successively fall out of equilibrium. However, there is a longstanding debate whether there is an underlying second-order phase transition in the hypothetical limit of infinitely long relaxation times.

Transition Temperature T_g

Refer to the figure on the right plotting the heat capacity as a function of temperature. In this context, T_g is the temperature corresponding to point A on the curve. The linear sections below and above T_g are colored green. T_g is the temperature at the intersection of the red regression lines.

Different operational definitions of the glass transition temperature T_g are in use, and several of them are endorsed as accepted scientific standards. Nevertheless, all definitions are arbitrary, and all yield different numeric results: at best, values of T_g for a given substance agree within a few kelvins. One definition refers to the viscosity, fixing T_g at a value of 10^{13} poise (or 10^{12} Pa·s). As evidenced experimentally, this value is close to the annealing point of many glasses.

In contrast to viscosity, the thermal expansion, heat capacity, and many other properties of inorganic glasses show a relatively sudden change at the glass transition temperature. Any such step or kink can be used to define T_g. To make this definition reproducible, the cooling or heating rate must be specified.

The most frequently used definition of T_g uses the energy release on heating in differential scanning calorimetry (DSC, see figure). Typically, the sample is first cooled with 10 K/min and then heated with that same speed.

Yet another definition of T_g uses the kink in dilatometry. Here, heating rates of 3-5 K/min are common. Summarized below are T_g values characteristic of certain classes of materials.

Nylon-6 Nylon has a glass transition temperature of 47 Celsius. Whereas Polyethene has a glass transition range of -130 to -80. It must be kept in mind that the above are only mean values, as the glass transition temperature depends on the cooling rate, molecular weight distribution and

could be influenced by additives. Note also that for a semi-crystalline material, such as polyethene that is 60-80% crystalline at room temperature, the quoted glass transition refers to what happens to the amorphous part of the material upon cooling.

In polymers the glass transition temperature, T_g, is often expressed as the temperature at which the Gibbs free energy is such that the activation energy for the cooperative movement of 50 or so elements of the polymer is exceeded. This allows molecular chains to slide past each other when a force is applied. From this definition, we can see that the introduction of relatively stiff chemical groups (such as benzene rings) will interfere with the flowing process and hence increase T_g.

The stiffness of thermoplastics decreases due to this effect (see Fig.19) When the glass temperature has been reached, the stiffness stays the same for a while, i.e., at or near E_2, until the temperature exceeds T_m, and the material melts. This region is called the rubber plateau.

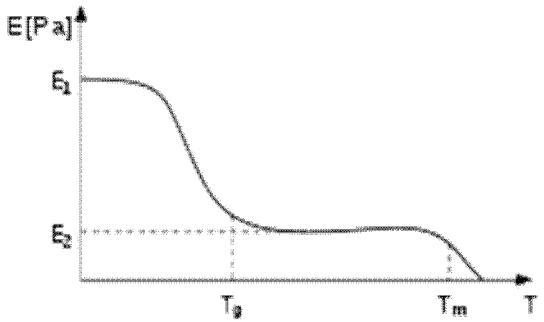


Fig.19 Stiffness versus temperature.

In ironing, a fabric is heated through this transition so that the polymer chains become mobile. The weight of the iron then imposes a preferred orientation. T_g can be significantly decreased by addition of plasticizers into the polymer matrix. Smaller molecules of plasticizer embed themselves between the polymer chains, increasing the spacing and free volume, and allowing them to move past one another even at lower temperatures. The "new-car smell" is due to the initial outgassing of volatile small-molecule plasticizers (most commonly known as phthalates) used to modify interior plastics (e.g., dashboards) to keep them from cracking in the cold of winter weather. The addition of nonreactive side groups to a polymer can also make the chains stand off from one another, reducing T_g. If a plastic with some desirable properties has a T_g which is too high, it can

sometimes be combined with another in a copolymer or composite material with a T_g below the temperature of intended use. Note that some plastics are used at high temperatures, e.g., in automobile engines, and others at low temperatures.

In viscoelastic materials, the presence of liquid-like behavior depends on the properties of and so varies with rate of applied load, i.e., how quickly a force is applied. The silicone toy 'Silly Putty' behaves quite differently depending on the time rate of applying a force: pull slowly and it flows, acting as a heavily viscous liquid; hit it with a hammer and it shatters, acting as a glass.

On cooling, rubber undergoes a liquid-glass transition, which has also been called a rubber-glass transition. For example, the Space Shuttle Challenger disaster was caused by rubber O-rings that were being used well below their glass transition temperature on an unusually cold Florida morning, and thus could not flex adequately to form proper seals between sections of the twosolid-fuel rocket boosters.

Dynamic Mechanical Analysis (abbreviated DMA, also known as **Dynamic Mechanical Spectroscopy**) is a technique used to study and characterize materials. It is most useful for studying the viscoelastic behavior of polymers. A sinusoidal stress is applied and the strain in the material is measured, allowing one to determine the complex modulus. The temperature of the sample or the frequency of the stress are often varied, leading to variations in the complex modulus; this approach can be used to locate the glass transition temperature of the material, as well as to identify transitions corresponding to other molecular motions.

One important application of DMA is measurement of the glass transition temperature of polymers. Amorphous polymers have different glass transition temperatures, above which the material will have rubbery properties instead of glassy behavior and the stiffness of the material will drop dramatically with an increase in viscosity. At the glass transition, the storage modulus decreases dramatically and the loss modulus reaches a maximum. Temperature-sweeping DMA is often used to characterize the glass transition temperature of a material.

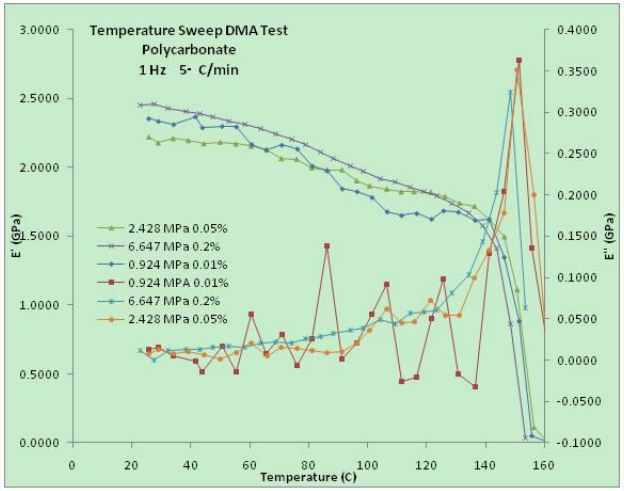


Fig. 20 A temperature sweep test on Polycarbonate. Storage Modulus (E') and Loss Modulus (E'') against temperature were plotted. Different initial static load and strain were used. The glass transition temperature of Polycarbonate was detected to be around 150°C.

12.7 Polymer Composition

Varying the composition of monomers and cross-linking can add or change the functionality of a polymer that can alter the results obtained from DMA. An example of such changes can be seen by blending ethylene-propylene-diene monomer (EPDM) with styrene-butadiene rubber (SBR) and different cross-linking or curing systems. Nair *et al*. abbreviate blends as E_0S, $E_{20}S$, etc., where E_0S equals the weight percent of EPDM in the blend and S denotes sulfur as the curing agent.

Increasing the amount of SBR in the blend decreased the storage modulus due to intermolecular and intramolecular interactions that can alter the physical state of the polymer. Within the glassy region, EPDM shows the highest storage modulus due to stronger intermolecular interactions (SBR has more steric hindrance that makes it less crystalline). In the rubbery region, SBR shows the highest storage modulus resulting from its ability to resist intermolecular slippage.

When compared to sulfur, the higher storage modulus occurred for blends cured with dicumyl peroxide (DCP) because of the relative strengths of C-C and C-S bonds.

Incorporation of reinforcing fillers into the polymer blends also increases the storage modulus at an expense of limiting the loss tangent peak height.

DMA can also be used to effectively evaluate the miscibility of polymers. The $E_{40}S$ blend had a much broader transition with a shoulder instead of a steep drop-off in a storage modulus plot of varying blend ratios, indicating that there are areas that are not homogeneous.

12.8 Instrumentation

The instrumentation of a DMA consists of a displacement sensor such as a linear variable differential transformer, which measures a change in voltage as a result of the instrument probe moving through a magnetic core, a temperature control system or furnace, a drive motor (a linear motor for probe loading which provides load for the applied force), a drive shaft support and guidance system to act as a guide for the force from the motor to the sample, and sample clamps in order to hold the sample being tested. Depending on what is being measured, samples will be prepared and handled differently. A general schematic of the primary components of a DMA instrument is shown in Figure 21.

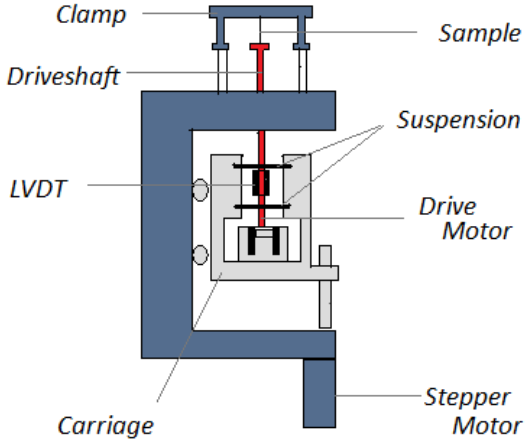


Fig. 21 General schematic of a DMA instrument.

13. POLYMER DEGRADATION

Aging of Polymers is the irreversible change in the properties of polymers under the action of, among other factors, heat, oxygen, sunlight, ozone, and ionizing radiation. Depending on the factor, the aging will be of the thermal, oxidative, light, ozone, or radiation type. Aging occurs during the storage and treatment of polymers, as well as during the storage and use of articles made from polymers. Under actual conditions, several factors act on polymers concurrently; for example, in atmospheric aging, oxygen, light, ozone, and moisture all affect the polymer. An important factor accelerating aging is mechanical stress that develops during the treatment of polymers and under certain operating conditions of polymer articles.

Aging is caused by chemical transformations of macromole-cules, which lead to the degradation of the macromolecules and the formation of branched or three-dimensional structures (cross-linking). There are various mechanisms of aging. For example, degradation in oxidative aging is related to a chain oxidation reaction of the polymer accompanied by the formation and decomposition of hydroperoxides. The rate of aging depends on the sensitivity of the polymer to the factors mentioned above, on the intensity of these factors, and on the composition of the polymer material. Carbon-chain polymers whose macromolecules contain unsaturated bonds, in particular, certain rubbers (natural rubber, synthetic isoprene rubbers), are most susceptible to aging.

Classification of the polymers aging processes:

Type of Aging	Activator	Accompanying Agent
thermal	heat	-
thermooxidative	heat	oxygen
light	light	oxygen
poisoning by metals	metall	oxygen
tiredness	mechanical impact	oxygen + deformation
atmosphere	ozone, oxygen, UV rays	tension
radiation	the emission of high energy	oxygen

Aging manifests itself in a deterioration of the mechanical characteristics of polymers, in the appearance and growth of cracks on the surface that sometimes result in destruction of the polymer, and in a change in color. The resistance of polymers to aging in many cases determines the storage period and, sometimes, the service life of polymer articles. Stabilization is an effective method for preventing aging in polymers.

Polymer Degradation is a change in the properties—tensile strength, colour, shape, etc.—of a polymer or polymer-based product under the influence of one or more environmental factors such as heat, light or chemicals such as acids, alkalis and some salts. These changes are usually undesirable, such as cracking and chemical disintegration of products or, more rarely, desirable, as inbiodegradation, or deliberately lowering the molecular weight of a polymer for recycling. The changes in properties are often termed "aging".

In a finished product such a change is to be prevented or delayed. Degradation can be useful for recycling/reusing the polymer waste to prevent or reduce environmental pollution. Degradation can also be induced deliberately to assist structure determination.

Polymeric molecules are very large (on the molecular scale), and their unique and useful properties are mainly a result of their size. Any loss in chain length lowers tensile strength and is a primary cause of premature cracking.

Commodity Polymers

Today there are primarily seven commodity polymers in use: polyethylene, polypropylene, polyvinyl chloride, polyethylene terephthalate, polystyrene, polycarbonate, and poly(methylmethacrylate) (Plexiglass). These make up nearly 98% of all polymers and plastics encountered in daily life. Each of these polymers has its own characteristic modes of degradation and resistances to heat, light and chemicals. Polyethylene, polypropylene, and poly(methylmethacrylate) are sensitive to oxidation and UV radiation, while PVC may discolour at high temperatures due to loss of hydrogen chloride gas, and become very brittle. PET is sensitive to hydrolysis and attack by strong acids, while polycarbonate depolymerizes rapidly when exposed to strong alkalis.

For example, polyethylene usually degrades by random scission - that is by a random breakage of the linkages (bonds) that hold the atoms of the polymer together. When this polymer is heated above 450 Celsius it

becomes a complex mixture of molecules of various sizes that resemble gasoline. Other polymers - like polyalphamethylstyrene - undergo 'unspecific' chain scission with breakage occurring only at the ends; they literally unzip or depolymerize to become the constituent monomers.

13.1 UV Degradation

Many natural and synthetic polymers are attacked by ultra-violet radiation and products made using these materials may crack or disintegrate (if they're not UV-stable). The problem is known as UV degradation, and is a common problem in products exposed to sunlight. Continuous exposure is a more serious problem than intermittent exposure, since attack is dependent on the extent and degree of exposure.

Many pigments and dyes can also be affected, when the problem is known as phototendering in textiles such as curtains or drapes.

Common synthetic polymers which may be attacked include polypropylene and LDPE where tertiary carbon bonds in their chain structures are the centres of attack. The ultra-violet rays activate such bonds to form free radicals, which then react further with oxygen in the atmosphere, producing carbonyl groups in the main chain. The exposed surfaces of products may then discolour and crack, although in bad cases, complete product disintegration can occur.

In fibre products like rope used in outdoor applications, product life will be low because the outer fibres will be attacked first, and will easily be damaged by abrasion for example. Discolouration of the rope may also occur, thus giving an early warning of the problem.

Polymers which possess UV-absorbing groups such as aromatic rings may also be sensitive to UV degradation. Aramid fibres like Kevlar for example are highly UV sensitive and must be protected from the deleterious effects of sunlight.

The problem can be detected before serious cracks are seen in a product using infra-red spectroscopy, where attack occurs by oxidation of bonds activated by the UV radiation forming carbonyl groups in the polymer chains.

In the example shown at left, carbonyl groups were easily detected by IR spectroscopy from a cast thin film. The product was a road cone made by rotational moulding in LDPE, which had cracked prematurely in service. Many similar cones also failed because an anti-UV additive had not been used during processing. Other plastic products which failed

included polypropylene mancabs used at roadworks which cracked after service of only a few months.

UV attack by sunlight can be ameliorated or prevented by adding anti-UV chemicals to the polymer when mixing the ingredients, prior to shaping the product by injection moulding for example. UV Stabilizers in plastics usually act by absorbing the UV radiation preferentially, and dissipating the energy as low level heat. The chemicals used are similar to those used in sunscreen cosmetic products, which protect skin from UV attack.

The effects of UV degradation on materials that require a long service life can be measured with accelerated exposure tests. With modern solar concentrator technologies, it is possible to simulate 63 years of natural UV radiation exposure on a test device in a single year. In Solar Power Modules degradation is a long-term issue according to material testings; the efficiency loss by UV degradation is about 6.81 to 9.54 % in 20 years.

13.2 Thermal Degradation of Polymers

Thermal degradation of polymers is molecular deterioration as a result of overheating. At high temperatures the components of the long chain backbone of the polymer can begin to separate (molecular scission) and react with one another to change the properties of the polymer. Thermal degradation can present an upper limit to the service temperature of plastics as much as the possibility of mechanical property loss. Indeed unless correctly prevented, significant thermal degradation can occur at temperatures much lower than those at which mechanical failure is likely to occur. The chemical reactions involved in thermal degradation lead to physical and optical property changes relative to the initially specified properties. Thermal degradation generally involves changes to themolecular weight (and molecular weight distribution) of the polymer and typical property changes include reduced ductility and embrittlement, chalking, color changes, cracking, general reduction in most other desirable physical properties.

Most types of degradation follow a similar basic pattern. The conventional model for thermal degradation is that of an autoxidation process which involves the major steps of initiation, propagation, branching, and termination.

Initiation

The initiation of thermal degradation involves the loss of a hydrogen atom from the polymer chain as a result of energy input from heat or

light. This creates a highly reactive and unstable polymer 'free radical' (R•) and a hydrogen atom with an unpaired electron (H•).

Propagation

The propagation of thermal degradation can involve a variety of reactions and one of these is where the free radical (R•) reacts with an oxygen (O_2) molecule to form a peroxy radical (ROO•) which can then remove a hydrogen atom from another polymer chain to form a hydroperoxide (ROOH) and so regenerate the free radical (R•). The hydroperoxide can then split into two new free radicals, (RO•) + (•OH), which will continue to propagate the reaction to other polymer molecules. The process can therefore accelerate depending on how easy it is to remove the hydrogen from the polymer chain.

Termination

The termination of thermal degradation is achieved by 'mopping up' the free radicals to create inert products. This can occur naturally by combining free radicals or it can be assisted by using stabilizersin the plastic.

(Thermogravimetric Analysis) (TGA) refers to the techniques where a sample is heated in a controlled atmosphere at a defined heating rate whilst the samples mass is measured. When a polymer sample degrades, its mass decreases due to the production of gaseous products like carbon monoxide, water vapour and carbon dioxide.

(Differential thermal analysis) (DTA) and (differential scanning calorimetry) (DSC): Analyzing the heating effect of polymer during the physical changes in terms of glass transition, melting, and so on. These techniques measure the heat flow associated with oxidation.

13.3 Ozonolysis

Cracks can be formed in many different elastomers by ozone attack. Tiny traces of the gas in the air will attack double bonds in rubber chains, with Natural rubber, polybutadiene, Styrene-butadiene rubber and NBR being most sensitive to degradation. Ozone cracks form in products under tension, but the critical strain is very small. The cracks are always oriented at right angles to the strain axis, so will form around the circumference in a rubber tube bent over. Such cracks are dangerous when they occur in fuel pipes because the cracks will grow from the outside exposed surfaces into the bore of the pipe, and fuel leakage and fire may follow. The problem

of ozone cracking can be prevented by adding anti-ozonants to the rubber before vulcanization. Ozone cracks were commonly seen in automobile tire sidewalls, but are now seen rarely thanks to these additives. On the other hand, the problem does recur in unprotected products such as rubber tubing and seals.

13.4 Stabilizers for Polymers

Stabilizers for polymers are used directly or by combinations to prevent the various effects such as oxidation, chain scission and uncontrolled recombinations and cross-linking reactions that are caused by photo-oxidation of polymers. Polymers are considered to get weathered due to the direct or indirect impact of heat and ultraviolet light. The effectiveness of the stabilizers against weathering depends on solubility, ability to stabilize in different polymer matrix, the distribution in matrix, evaporation loss during processing and use. The effect on the viscosity is also an important concern for processing.

Heat stabilizers are mainly used for construction products made of polyvinyl chloride, for instance window profiles, pipes and cable ducts. Light stabilizers, for instance HALS, are especially needed for polypropylene and polyethylene. The environmental impact of stabilizers for polymers can be problematic because of heavy metal content. In Europe lead stabilizers are increasingly replaced by other types, for example calcium-zinc stabilizers.

Antioxidants are used to terminate the oxidation reactions taking place due to different weathering conditions and reduce the degradation of organic materials. For example, synthetic polymers react with atmospheric oxygen. Organic materials undergo auto-oxidizations due to free radical chain reaction. Oxidatively sensitive substrates will react with atmospheric oxygen directly and produce free radicals. Free radicals are of different forms, consider organic material RH. This material reacts with oxygen to give free radicals such as R• , RO• , ROO• , HO•. These free radicals further react with atmospheric oxygen to produce more and more free radicals. For example, R• + O2 → ROO• ROO• + RH → ROOH + R•. This can be terminated using the antioxidants. Then this reaction comes to, 2R• → R---- R ROO• + R• → ROOR 2ROO• → Non-radical products. Weathering of polymers is caused by absorption of UV lights, which results in, radical initiated auto-oxidation. This produces cleavage of hydro peroxides and carbonyl compounds. This is because of the weak bond in hydro peroxides

which is the main source for the free radicals to initiate from. Homolytic decomposition of hydro peroxide increases the rate of free radicals production. Therefore it is important factor in determining oxidative stability. The conversion of peroxy and alkyl radicals to non-radical species terminates the chain reaction, thereby decreasing the kinetic chain length.

Hydrogen-donating antioxidants (AH), such as hindered phenols and secondary aromatic amines, inhibit oxidation by competing with organic substrate (RH) for peroxy radicals, thereby terminating the chain reaction and stabilizing the further oxidation reactions.

At K17, ROO• + AH → ROOH + A•.

At K6, ROO• + RH → ROOH + R•.

Here K17 is larger than K6, therefore AH can be at low concentrations. At low concentrations AH are more effective because the usual concentration in saturated plastic polymer range from 0.01 to 0.05% based on the weight of the polymer.

Benzofuranones is another most effective antioxidant, which terminates the chain reaction by donating weakly bonded benzylic hydrogen atom and gets reduced to a stable benzofuranyl (lactone).

Antioxidants inhibit the formation of the free radicals thereby enhancing the stability of polymers against light and heat.

The ability of hindered amine light stabilisers (HALS) to scavenge radicals which are produced by weathering, may be explained by the formation of nitroxyl radicals through a process known as the Denisov Cycle. The nitroxyl radical(R-O•) combines with free radicals in polymers:

R-O• + R'• → R-O-R'

Although they are traditionally considered as light stabilizers, they can also stabilize thermal degradation.

Even though HALS are extremely effective in polyolefins, polyethylene and polyurethane, they are ineffective in polyvinyl chloride (PVC). It is thought that their ability to form nitroxyl radicals is disrupted. HALS act as a base and become neutralized by hydrochloric acid (HCl) that is released by photooxidation of PVC. The exception is the recently developed NOR HALS which is not a strong base and is not deactivated by HCl.

UV Absorber

The UV absorbers dissipate the absorbed light energy from UV rays as heat by reversible intramolecular proton transfer. This reduces the absorption of UV rays by the polymer matrix and hence reduces the rate of

weathering. Typical UV-absorbers are oxanilides for polyamides, benzophenones for PVC, benzotriazoles and hydroxyphenyltriazines for polycarbonate.

Strongly light-absorbing PPS is difficult to stabilize. Even antioxidants fail in this polymer since the polymer is electron-rich and behaves as antioxidant. The acids or bases in the PPS matrix can disrupt the performance of the conventional UV absorbers such as HPBT. PTHPBT which is modification of HPBT are shown to be effective even in these conditions.

Antiozonants

An **Antiozonant**, also known as **Anti-Ozonant**, is a chemical compound that prevents or slows down the degradation of material caused by ozone gas in the air (ozone cracking). Antiozonants are used as additives to plastics and rubber, especially in tire manufacturing.

Common antiozonants include:

- Paraffin waxes that form a surface barrier
- *p*-Phenylenediamines such as 6PPP (*N*-(1,3-dimethylbutyl)-*N*'-phenyl-*p*-phenylenediamine) or IPPD (*N*-isopropyl-*N*'-phenyl-*p*-phenylenediamine)
- Ethylene diurea (EDU)

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