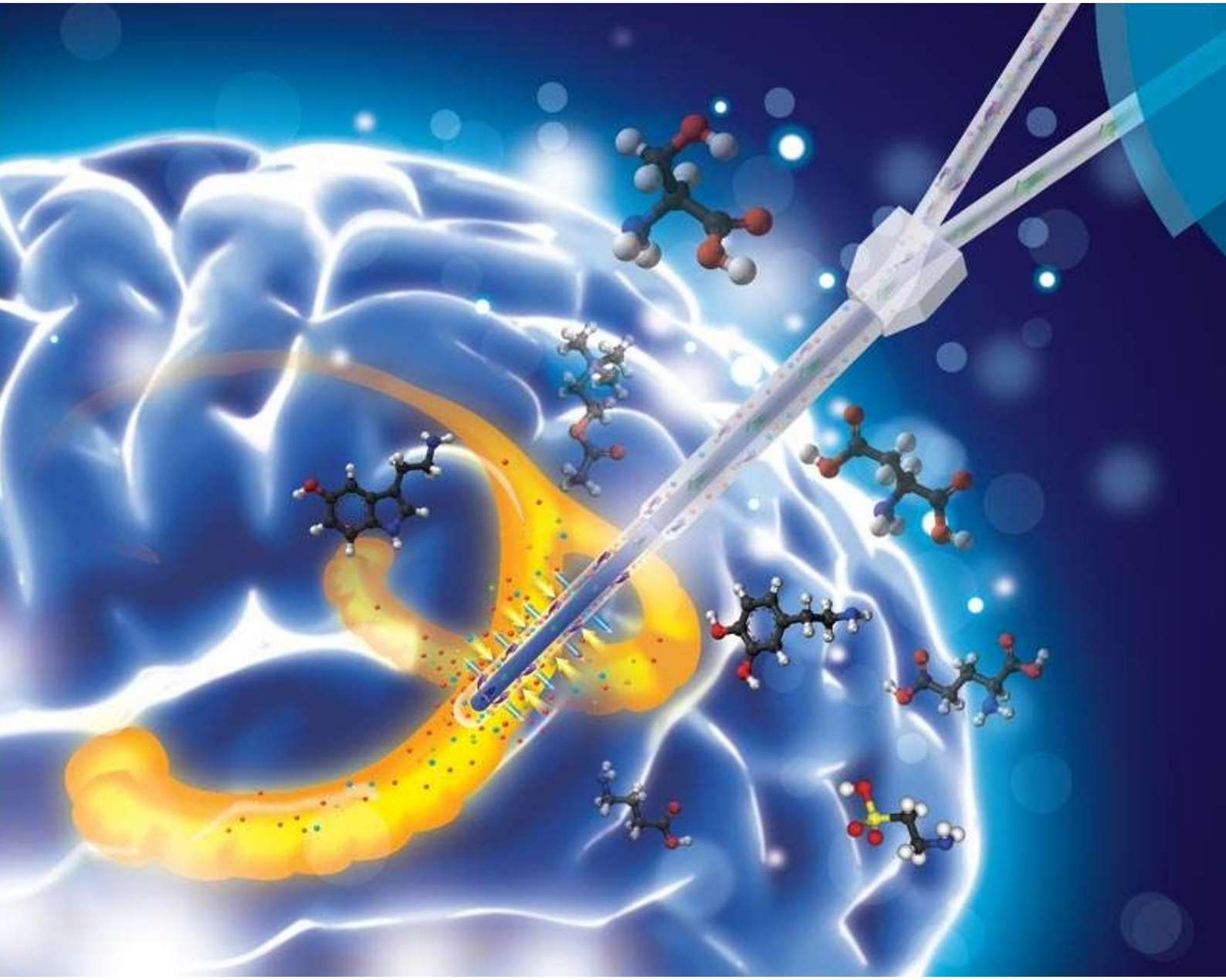
Selecting Method of Analytical Chemistry

Eric Becker



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by Eric Becker

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Chapter 1

Analytical Chemistry

Analytical chemistry studies and uses instruments and methods used to separate, identify, and quantify matter. In practice, separation, identification or quantification may constitute the entire analysis or be combined with another method. Separation isolates analytes. Qualitative analysis identifies analytes, while quantitative analysis determines the numerical amount or concentration. Analytical chemistry is the **science of obtaining, processing, and communicating information about the composition and structure of matter**. In other words, it is the art and science of determining what matter is and how much of it exists. ... It is one of the most popular fields of work for ACS chemists.

Analytical chemistry consists of classical, wet chemical methods and modern, instrumental methods. Classical qualitative methods use separations such as precipitation, extraction, and distillation. Identification may be based on differences in color, odor, melting point, boiling point, solubility, radioactivity or reactivity. Classical quantitative analysis uses mass or volume changes to quantify amount. Instrumental methods may be used to separate samples using chromatography, electrophoresis or field flow fractionation. Then qualitative and quantitative analysis can be performed, often with the same instrument and may use light interaction, heat interaction, electric fields or magnetic fields. Often the same instrument can separate, identify and quantify an analyte.

Analytical chemistry is also focused on improvements in experimental design, chemometrics, and the creation of new measurement tools. Analytical chemistry has broad applications to medicine, science and engineering.

History

Analytical chemistry has been important since the early days of chemistry, providing methods for determining which elements and chemicals are present in the object in question. During this period, significant contributions to analytical chemistry included the development of systematic elemental analysis by Justus von Liebig and systematized organic analysis based on the specific reactions of functional groups.

The first instrumental analysis was flame emissive spectrometry developed by Robert Bunsen and Gustav Kirchhoff who discovered rubidium (Rb) and caesium (Cs) in 1860.

Most of the major developments in analytical chemistry take place after 1900. During this period instrumental analysis becomes progressively dominant in the field. In particular many of the basic spectroscopic and spectrometric techniques were discovered in the early 20th century and refined in the late 20th century.

The separation sciences follow a similar time line of development and also become increasingly transformed into high performance instruments. In the 1970s many of these techniques began to be used together as hybrid techniques to achieve a complete characterization of samples.

Starting in approximately the 1970s into the present day analytical chemistry has progressively become more inclusive of biological questions (bioanalytical chemistry), whereas it had previously been largely focused on inorganic or small organic molecules. Lasers have been increasingly used in chemistry as probes and even to initiate and influence a wide variety of reactions. The late 20th century also saw an expansion of the application of analytical chemistry from somewhat academic chemical questions to forensic, environmental, industrial and medical questions, such as in histology.

Modern analytical chemistry is dominated by instrumental analysis. Many analytical chemists focus on a single type of instrument.

Academics tend to either focus on new applications and discoveries or on new methods of analysis. The discovery of a chemical present in blood that increases the risk of cancer would be a discovery that an analytical chemist might be involved in. An effort to develop a new method might involve the use of a tunable laser to increase the specificity and sensitivity of a spectrometric method. Many methods, once developed, are kept purposely static so that data can be compared over long periods of time. This is particularly true in industrial quality assurance (QA), forensic and environmental applications. Analytical chemistry plays an increasingly important role in the pharmaceutical industry where, aside from QA, it is used in discovery of new drug candidates and in clinical applications where understanding the interactions between the drug and the patient are critical.

Classical methods

Although modern analytical chemistry is dominated by sophisticated instrumentation, the roots of analytical chemistry and some of the principles used in modern instruments are from traditional techniques, many of which are still used today. These techniques also tend to form the backbone of most undergraduate analytical chemistry educational labs.

Qualitative analysis

A qualitative analysis determines the presence or absence of a particular compound, but not the mass or concentration. By definition, qualitative analyses do not measure quantity.

Chemical tests

There are numerous qualitative chemical tests, for example, the acid test for gold and the Kastle-Meyer test for the presence of blood.

Flame test

Inorganic qualitative analysis generally refers to a systematic scheme to confirm the presence of certain aqueous ions or elements by performing a series of reactions that eliminate ranges of possibilities and then confirms suspected ions with a confirming test. Sometimes small carbon containing ions are included in such schemes. With modern instrumentation these tests are rarely used but can be useful for educational

purposes and in field work or other situations where access to state-of-the-art instruments are not available or expedient.

Quantitative analysis

Quantitative analysis is the measurement of the quantities of particular chemical constituents present in a substance. Quantities can be measured by mass (gravimetric analysis) or volume (volumetric analysis).

Gravimetric analysis

Gravimetric analysis involves determining the amount of material present by weighing the sample before and/or after some transformation. A common example used in undergraduate education is the determination of the amount of water in a hydrate by heating the sample to remove the water such that the difference in weight is due to the loss of water.

Volumetric analysis

Titration involves the addition of a reactant to a solution being analyzed until some equivalence point is reached. Often the amount of material in the solution being analyzed may be determined. Most familiar to those who have taken chemistry during secondary education is the acid-base titration involving a color changing indicator. There are many other types of titrations, for example potentiometric titrations. These titrations may use different types of indicators to reach some equivalence point.

Instrumental methods

Spectroscopy

Spectroscopy measures the interaction of the molecules with electromagnetic radiation. Spectroscopy consists of many different applications such as atomic absorption spectroscopy, atomic emission spectroscopy, ultraviolet-visible spectroscopy, x-ray spectroscopy, fluorescence spectroscopy, infrared spectroscopy, Raman spectroscopy, dual polarization interferometry, nuclear magnetic resonance spectroscopy, photoemission spectroscopy, Mössbauer spectroscopy and so on.

Mass spectrometry

Mass spectrometry measures mass-to-charge ratio of molecules using electric and magnetic fields. There are several ionization methods: electron ionization, chemical ionization, electrospray ionization, fast atom bombardment, matrix assisted laser desorption/ionization, and others. Also, mass spectrometry is categorized by approaches of mass analyzers: magnetic-sector, quadrupole mass analyzer, quadrupole ion trap, time-of-flight, Fourier transform ion cyclotron resonance, and so on.

Electrochemical analysis

Electroanalytical methods measure the potential (volts) and/or current (amps) in an electrochemical cell containing the analyte. These methods can be categorized according to which aspects of the cell are controlled and which are measured. The

four main categories are potentiometry (the difference in electrode potentials is measured), coulometry (the transferred charge is measured over time), amperometry (the cell's current is measured over time), and voltammetry (the cell's current is measured while actively altering the cell's potential).

Thermal analysis

Calorimetry and thermogravimetric analysis measure the interaction of a material and heat.

Separation

Separation processes are used to decrease the complexity of material mixtures. Chromatography, electrophoresis and field flow fractionation are representative of this field.

Hybrid techniques

Combinations of the above techniques produce a "hybrid" or "hyphenated" technique. Several examples are in popular use today and new hybrid techniques are under development. For example, gas chromatography-mass spectrometry, gas chromatography-infrared spectroscopy, liquid chromatography-mass spectrometry, liquid chromatography-NMR spectroscopy. liquidchromagraphy-infrared spectroscopy and capillary electrophoresis-mass spectrometry.

Hyphenated separation techniques refers to a combination of two (or more) techniques to detect and separate chemicals from solutions. Most often the other technique is some form of chromatography. Hyphenated techniques are widely used in

chemistry and biochemistry. A slashis sometimes used instead of hyphen, especially if the name of one of the methods contains a hyphen itself.

Microscopy

The visualization of single molecules, single cells, biological tissues and nanomaterials is an important and attractive approach in analytical science. Also, hybridization with other traditional analytical tools is revolutionizing analytical science. Microscopycan be categorized into three different fields: optical microscopy, electron microscopy, and scanning probe microscopy. Recently, this field is rapidly progressing because of the rapid development of the computer and camera industries.

Lab-on-a-chip

Devices that integrate (multiple) laboratory functions on a single chip of only millimeters to a few square centimeters in size and that are capable of handling extremely small fluid volumes down to less than picoliters.

Errors

- Error can be defined as numerical difference between observed value and true value. The experimental error can be divided into two types, systematic error and random error. Systematic error results from a flaw in equipment or the design of an experiment while random error results from uncontrolled or uncontrollable variables in the experiment.

Standards

Standard curve

A general method for analysis of concentration involves the creation of a calibration curve. This allows for determination of the amount of a chemical in a material by comparing the results of unknown sample to those of a series of known standards. If the concentration of element or compound in a sample is too high for the detection range of the technique, it can simply be diluted in a pure solvent. If the amount in the sample is below an instrument's range of measurement, the method of addition can be used. In this method a known quantity of the element or compound under study is added, and the difference between the concentration added, and the concentration observed is the amount actually in the sample.

Internal standards

Sometimes an internal standardis added at a known concentration directly to an analytical sample to aid in quantitation. The amount of analyte present is then determined relative to the internal standard as a calibrant. An ideal internal standard is isotopically-enrichedanalyte which gives rise to the method of isotope dilution.

Standard addition

The method of standard additionis used in instrumental analysis to determine concentration of a substance (analyte) in an unknown sample by comparison to a set of samples of

known concentration, similar to using a calibration curve. Standard addition can be applied to most analytical techniques and is used instead of a calibration curve to solve the matrix effect problem.

Signals and noise

One of the most important components of analytical chemistry is maximizing the desired signal while minimizing the associated noise. The analytical figure of merit is known as the signal-to-noise ratio (S/N or SNR).

Noise can arise from environmental factors as well as from fundamental physical processes.

Flicker noise

Flicker noise is electronic noise with a $1/f$ frequency spectrum; as f increases, the noise decreases. Flicker noise arises from a variety of sources, such as impurities in a conductive channel, generation and recombination noise in a transistor due to base current, and so on. This noise can be avoided by modulation of the signal at a higher frequency, for example through the use of a lock-in amplifier.

Environmental noise

Environmental noise arises from the surroundings of the analytical instrument. Sources of electromagnetic noise are power lines, radio and television stations, wireless devices, compact fluorescent lamps and electric motors. Many of these noise sources are narrow bandwidth and therefore can be

avoided. Temperature and vibration isolation may be required for some instruments.

Noise reduction

Noise reduction can be accomplished either in computer hardware or software. Examples of hardware noise reduction are the use of shielded cable, analog filtering, and signal modulation. Examples of software noise reduction are digital filtering, ensemble average, boxcar average, and correlation methods.

Applications

Analytical chemistry has applications including in forensic science, bioanalysis, clinical analysis, environmental analysis, and materials analysis. Analytical chemistry research is largely driven by performance (sensitivity, detection limit, selectivity, robustness, dynamic range, linear range, accuracy, precision, and speed), and cost (purchase, operation, training, time, and space). Among the main branches of contemporary analytical atomic spectrometry, the most widespread and universal are optical and mass spectrometry. In the direct elemental analysis of solid samples, the new leaders are laser-induced breakdown and laser ablation mass spectrometry, and the related techniques with transfer of the laser ablation products into inductively coupled plasma. Advances in design of diode lasers and optical parametric oscillators promote developments in fluorescence and ionization spectrometry and also in absorption techniques where uses of optical cavities for increased effective absorption pathlength are expected to

expand. The use of plasma- and laser-based methods is increasing. An interest towards absolute (standardless) analysis has revived, particularly in emission spectrometry.

Great effort is being put in shrinking the analysis techniques to chip size. Although there are few examples of such systems competitive with traditional analysis techniques, potential advantages include size/portability, speed, and cost. (micrototal analysis system (μTAS) or lab-on-a-chip). Microscale chemistry reduces the amounts of chemicals used.

Many developments improve the analysis of biological systems. Examples of rapidly expanding fields in this area are genomics, DNA sequencing and related research in genetic fingerprinting and DNA microarray; proteomics, the analysis of protein concentrations and modifications, especially in response to various stressors, at various developmental stages, or in various parts of the body, metabolomics, which deals with metabolites; transcriptomics, including mRNA and associated fields; lipidomics - lipids and its associated fields; peptidomics - peptides and its associated fields; and metalomics, dealing with metal concentrations and especially with their binding to proteins and other molecules.

Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, forensic science and so on.

The recent developments of computer automation and information technologies have extended analytical chemistry into a number of new biological fields. For example, automated

DNA sequencing machines were the basis to complete human genome projects leading to the birth of genomics. Protein identification and peptide sequencing by mass spectrometry opened a new field of proteomics. In addition to automating specific processes, there is effort to automate larger sections of lab testing, such as in companies like Emerald Cloud Lab and Transcriptic.

Analytical chemistry has been an indispensable area in the development of nanotechnology. Surface characterization instruments, electron microscopes and scanning probe microscopes enables scientists to visualize atomic structures with chemical characterizations.

Chapter 2

Instrumentation

Atomic absorption spectroscopy

Atomic absorption spectroscopy (**AAS**) and atomic emission spectroscopy (**AES**) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions.

In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution, or directly in solid samples via electrothermal vaporization, and is used in pharmacology, biophysics, archaeology and toxicology research.

Atomic emission spectroscopy was first used as an analytical technique, and the underlying principles were established in the second half of the 19th century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg, Germany.

The modern form of AAS was largely developed during the 1950s by a team of Australian chemists. They were led by Sir Alan Walsh at the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Chemical Physics, in Melbourne, Australia.

Atomic absorption spectrometry has many uses in different areas of chemistry such as clinical analysis of metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, hair, muscle tissue. Atomic absorption spectrometry can be used in qualitative and quantitative analysis.

Principles

The technique makes use of the atomic absorption spectrum of a sample in order to assess the concentration of specific analytes within it. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer–Lambert law.

Instrumentation

In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electrothermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element-specific line radiation source or a continuum radiation source.

The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

Atomizers

The atomizers most commonly used nowadays are (spectroscopic) flames and electrothermal (graphite tube) atomizers. Other atomizers, such as glow-discharge atomization, hydride atomization, or cold-vapor atomization, might be used for special purposes.

Flame atomizers

- The oldest and most commonly used atomizers in AAS are flames, principally the air-acetylene flame with a temperature of about 2300 °C and the nitrous oxide system (N_2O)-acetylene flame with a temperature of about 2700 °C. The latter flame, in addition, offers a more reducing environment, being ideally suited for analytes with high affinity to oxygen.

Liquid or dissolved samples are typically used with flame atomizers. The sample solution is aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which is introduced into a spray chamber, where it is mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (< 10 μm) enter the flame. This conditioning process reduces interference, but only about 5% of the aerosolized solution reaches the flame because of it.

On top of the spray chamber is a burner head that produces a flame that is laterally long (usually 5–10 cm) and only a few mm deep. The radiation beam passes through this flame at its longest axis, and the flame gas flow-rates may be adjusted to

produce the highest concentration of free atoms. The burner height may also be adjusted, so that the radiation beam passes through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

The processes in a flame include the stages of desolvation (drying) in which the solvent is evaporated and the dry sample nano-particles remain, vaporization (transfer to the gaseous phase) in which the solid particles are converted into gaseous molecule, atomization in which the molecules are dissociated into free atoms, and ionization where (depending on the ionization potential of the analyte atoms and the energy available in a particular flame) atoms may be in part converted to gaseous ions.

Each of these stages includes the risk of interference in case the degree of phase transfer is different for the analyte in the calibration standard and in the sample. Ionization is generally undesirable, as it reduces the number of atoms that are available for measurement, i.e., the sensitivity.

In flame AAS a steady-state signal is generated during the time period when the sample is aspirated. This technique is typically used for determinations in the mg L range, and may be extended down to a few μg L for some elements.

Electrothermal atomizers

Electrothermal AAS (ET AAS) using graphite tube atomizers was pioneered by Boris V. L'vov at the Saint Petersburg Polytechnical Institute, Russia, since the late 1950s, and investigated in parallel by Hans Massmann at the Institute of

Spectrochemistry and Applied Spectroscopy (ISAS) in Dortmund, Germany.

Although a wide variety of graphite tube designs have been used over the years, the dimensions nowadays are typically 20–25 mm in length and 5–6 mm inner diameter. With this technique liquid/dissolved, solid and gaseous samples may be analyzed directly. A measured volume (typically 10–50 μL) or a weighed mass (typically around 1 mg) of a solid sample are introduced into the graphite tube and subject to a temperature program. This typically consists of stages, such as drying – the solvent is evaporated; pyrolysis – the majority of the matrix constituents are removed; atomization – the analyte element is released to the gaseous phase; and cleaning – eventual residues in the graphite tube are removed at high temperature.

The graphite tubes are heated via their ohmic resistance using a low-voltage high-current power supply; the temperature in the individual stages can be controlled very closely, and temperature ramps between the individual stages facilitate separation of sample components. Tubes may be heated transversely or longitudinally, where the former ones have the advantage of a more homogeneous temperature distribution over their length. The so-called stabilized temperature platform furnace (STPF) concept, proposed by Walter Slavin, based on research of Boris L'vov, makes ET AAS essentially free from interference. The major components of this concept are atomization of the sample from a graphite platform inserted into the graphite tube (L'vov platform) instead of from the tube wall in order to delay atomization until the gas phase in the atomizer has reached a stable temperature; use of a chemical modifier in order to stabilize the analyte to a pyrolysis

temperature that is sufficient to remove the majority of the matrix components; and integration of the absorbance over the time of the transient absorption signal instead of using peak height absorbance for quantification.

In ET AAS a transient signal is generated, the area of which is directly proportional to the mass of analyte (not its concentration) introduced into the graphite tube. This technique has the advantage that any kind of sample, solid, liquid or gaseous, can be analyzed directly. Its sensitivity is 2–3 orders of magnitude higher than that of flame AAS, so that determinations in the low µg L range (for a typical sample volume of 20 µL) and ng g range (for a typical sample mass of 1 mg) can be carried out. It shows a very high degree of freedom from interferences, so that ET AAS might be considered the most robust technique available nowadays for the determination of trace elements in complex matrices.

Specialized atomization techniques

While flame and electrothermal vaporizers are the most common atomization techniques, several other atomization methods are utilized for specialized use.

Glow-discharge atomization

A glow-discharge device (GD) serves as a versatile source, as it can simultaneously introduce and atomize the sample. The glow discharge occurs in a low-pressure argon gas atmosphere between 1 and 10 torr. In this atmosphere lies a pair of electrodes applying a DC voltage of 250 to 1000 V to break down the argon gas into positively charged ions and electrons.

These ions, under the influence of the electric field, are accelerated into the cathode surface containing the sample, bombarding the sample and causing neutral sample atom ejection through the process known as sputtering. The atomic vapor produced by this discharge is composed of ions, ground state atoms, and fraction of excited atoms. When the excited atoms relax back into their ground state, a low-intensity glow is emitted, giving the technique its name.

The requirement for samples of glow discharge atomizers is that they are electrical conductors. Consequently, atomizers are most commonly used in the analysis of metals and other conducting samples. However, with proper modifications, it can be utilized to analyze liquid samples as well as nonconducting materials by mixing them with a conductor (e.g. graphite).

Hydride atomization

Hydride generation techniques are specialized in solutions of specific elements. The technique provides a means of introducing samples containing arsenic, antimony, selenium, bismuth, and lead into an atomizer in the gas phase. With these elements, hydride atomization enhances detection limits by a factor of 10 to 100 compared to alternative methods. Hydride generation occurs by adding an acidified aqueous solution of the sample to a 1% aqueous solution of sodium borohydride, all of which is contained in a glass vessel. The volatile hydride generated by the reaction that occurs is swept into the atomization chamber by an inert gas, where it undergoes decomposition. This process forms an atomized form of the analyte, which can then be measured by absorption or emission spectrometry.

Cold-vapor atomization

The cold-vapor technique is an atomization method limited to only the determination of mercury, due to it being the only metallic element to have a large enough vapor pressure at ambient temperature. Because of this, it has an important use in determining organic mercury compounds in samples and their distribution in the environment. The method initiates by converting mercury into Hg by oxidation from nitric and sulfuric acids, followed by a reduction of Hg with tin(II) chloride. The mercury, is then swept into a long-pass absorption tube by bubbling a stream of inert gas through the reaction mixture. The concentration is determined by measuring the absorbance of this gas at 253.7 nm. Detection limits for this technique are in the parts-per-billion range making it an excellent mercury detection atomization method.

Two types of burners are used: total consumption burner and premix burner.

Radiation sources

- We have to distinguish between line source AAS (LS AAS) and continuum source AAS (CS AAS). In classical LS AAS, as it has been proposed by Alan Walsh, the high spectral resolution required for AAS measurements is provided by the radiation source itself that emits the spectrum of the analyte in the form of lines that are narrower than the absorption lines. Continuum sources, such as deuterium lamps, are only used for background correction purposes. The advantage of this technique is that only a

medium-resolution monochromator is necessary for measuring AAS; however, it has the disadvantage that usually a separate lamp is required for each element that has to be determined. In CS AAS, in contrast, a single lamp, emitting a continuum spectrum over the entire spectral range of interest is used for all elements. Obviously, a high-resolution monochromator is required for this technique, as will be discussed later.

Hollow cathode lamps

Hollow cathode lamps (HCL) are the most common radiation source in LS AAS. Inside the sealed lamp, filled with argon or neon gas at low pressure, is a cylindrical metal cathode containing the element of interest and an anode. A high voltage is applied across the anode and cathode, resulting in an ionization of the fill gas. The gas ions are accelerated towards the cathode and, upon impact on the cathode, sputter cathode material that is excited in the glow discharge to emit the radiation of the sputtered material, i.e., the element of interest. In the majority of cases single element lamps are used, where the cathode is pressed out of predominantly compounds of the target element. Multi-element lamps are available with combinations of compounds of the target elements pressed in the cathode. Multi element lamps produce slightly less sensitivity than single element lamps and the combinations of elements have to be selected carefully to avoid spectral interferences. Most multi-element lamps combine a handful of elements, e.g.: 2 - 8. Atomic Absorption Spectrometers can feature as few as 1-2 hollow cathode lamp

positions or in automated multi-element spectrometers, a 8-12 lamp positions may be typically available.

Electrodeless discharge lamps

Electrodeless discharge lamps (EDL) contain a small quantity of the analyte as a metal or a salt in a quartz bulb together with an inert gas, typically argon gas, at low pressure. The bulb is inserted into a coil that is generating an electromagnetic radio frequency field, resulting in a low-pressure inductively coupled discharge in the lamp. The emission from an EDL is higher than that from an HCL, and the line width is generally narrower, but EDLs need a separate power supply and might need a longer time to stabilize.

Deuterium lamps

- Deuterium HCL or even hydrogen HCL and deuterium discharge lamps are used in LS AAS for background correction purposes. The radiation intensity emitted by these lamps decreases significantly with increasing wavelength, so that they can be only used in the wavelength range between 190 and about 320 nm.

Continuum sources

When a continuum radiation source is used for AAS, it is necessary to use a high-resolution monochromator, as will be discussed later. In addition, it is necessary that the lamp emits radiation of intensity at least an order of magnitude above that of a typical HCL over the entire wavelength range from 190 nm

to 900 nm. A special high-pressure xenon short arc lamp, operating in a hot-spot mode has been developed to fulfill these requirements.

Spectrometer

As already pointed out above, there is a difference between medium-resolution spectrometers that are used for LS AAS and high-resolution spectrometers that are designed for CS AAS. The spectrometer includes the spectral sorting device (monochromator) and the detector.

Spectrometers for LS AAS

In LS AAS the high resolution that is required for the measurement of atomic absorption is provided by the narrow line emission of the radiation source, and the monochromator simply has to resolve the analytical line from other radiation emitted by the lamp. This can usually be accomplished with a band pass between 0.2 and 2 nm, i.e., a medium-resolution monochromator. Another feature to make LS AAS element-specific is modulation of the primary radiation and the use of a selective amplifier that is tuned to the same modulation frequency, as already postulated by Alan Walsh. This way any (unmodulated) radiation emitted for example by the atomizer can be excluded, which is imperative for LS AAS. Simple monochromators of the Littrow or (better) the Czerny-Turner design are typically used for LS AAS. Photomultiplier tubes are the most frequently used detectors in LS AAS, although solid state detectors might be preferred because of their better signal-to-noise ratio.

Spectrometers for CS AAS

When a continuum radiation source is used for AAS measurement it is indispensable to work with a high-resolution monochromator. The resolution has to be equal to or better than the half width of an atomic absorption line (about 2 pm) in order to avoid losses of sensitivity and linearity of the calibration graph. The research with high-resolution (HR) CS AAS was pioneered by the groups of O'Haver and Harnly in the US, who also developed the (up until now) only simultaneous multi-element spectrometer for this technique. The breakthrough, however, came when the group of Becker-Ross in Berlin, Germany, built a spectrometer entirely designed for HR-CS AAS. The first commercial equipment for HR-CS AAS was introduced by Analytik Jena (Jena, Germany) at the beginning of the 21st century, based on the design proposed by Becker-Ross and Florek. These spectrometers use a compact double monochromator with a prism pre-monochromator and an echelle grating monochromator for high resolution. A linear charge-coupled device (CCD) array with 200 pixels is used as the detector. The second monochromator does not have an exit slit; hence the spectral environment at both sides of the analytical line becomes visible at high resolution. As typically only 3–5 pixels are used to measure the atomic absorption, the other pixels are available for correction purposes. One of these corrections is that for lamp flicker noise, which is independent of wavelength, resulting in measurements with very low noise level; other corrections are those for background absorption, as will be discussed later.

Background absorption and background correction

The relatively small number of atomic absorption lines (compared to atomic emission lines) and their narrow width (a few pm) make spectral overlap rare; there are only few examples known that an absorption line from one element will overlap with another. Molecular absorption, in contrast, is much broader, so that it is more likely that some molecular absorption band will overlap with an atomic line. This kind of absorption might be caused by un-dissociated molecules of concomitant elements of the sample or by flame gases. We have to distinguish between the spectra of di-atomic molecules, which exhibit a pronounced fine structure, and those of larger (usually tri-atomic) molecules that don't show such fine structure. Another source of background absorption, particularly in ET AAS, is scattering of the primary radiation at particles that are generated in the atomization stage, when the matrix could not be removed sufficiently in the pyrolysis stage.

All these phenomena, molecular absorption and radiation scattering, can result in artificially high absorption and an improperly high (erroneous) calculation for the concentration or mass of the analyte in the sample. There are several techniques available to correct for background absorption, and they are significantly different for LS AAS and HR-CS AAS.

Background correction techniques in LS AAS

In LS AAS background absorption can only be corrected using instrumental techniques, and all of them are based on two

sequential measurements: firstly, total absorption (atomic plus background), secondly, background absorption only. The difference of the two measurements gives the net atomic absorption. Because of this, and because of the use of additional devices in the spectrometer, the signal-to-noise ratio of background-corrected signals is always significantly inferior compared to uncorrected signals. It should also be pointed out that in LS AAS there is no way to correct for (the rare case of) a direct overlap of two atomic lines. In essence there are three techniques used for background correction in LS AAS:

Deuterium background correction

This is the oldest and still most commonly used technique, particularly for flame AAS. In this case, a separate source (a deuterium lamp) with broad emission is used to measure the background absorption over the entire width of the exit slit of the spectrometer. The use of a separate lamp makes this technique the least accurate one, as it cannot correct for any structured background. It also cannot be used at wavelengths above about 320 nm, as the emission intensity of the deuterium lamp becomes very weak. The use of deuterium HCL is preferable compared to an arc lamp due to the better fit of the image of the former lamp with that of the analyte HCL.

Smith-Hieftje background correction

This technique (named after their inventors) is based on the line-broadening and self-reversal of emission lines from HCL when high current is applied. Total absorption is measured with normal lamp current, i.e., with a narrow emission line, and background absorption after application of a high-current

pulse with the profile of the self-reversed line, which has little emission at the original wavelength, but strong emission on both sides of the analytical line. The advantage of this technique is that only one radiation source is used; among the disadvantages are that the high-current pulses reduce lamp lifetime, and that the technique can only be used for relatively volatile elements, as only those exhibit sufficient self-reversal to avoid dramatic loss of sensitivity. Another problem is that background is not measured at the same wavelength as total absorption, making the technique unsuitable for correcting structured background.

Zeeman-effect background correction

An alternating magnetic field is applied at the atomizer (graphite furnace) to split the absorption line into three components, the π component, which remains at the same position as the original absorption line, and two σ components, which are moved to higher and lower wavelengths, respectively. Total absorption is measured without magnetic field and background absorption with the magnetic field on. The π component has to be removed in this case, e.g. using a polarizer, and the σ components do not overlap with the emission profile of the lamp, so that only the background absorption is measured.

The advantages of this technique are that total and background absorption are measured with the same emission profile of the same lamp, so that any kind of background, including background with fine structure can be corrected accurately, unless the molecule responsible for the background is also affected by the magnetic field and using a chopper as a

polariser reduces the signal to noise ratio. While the disadvantages are the increased complexity of the spectrometer and power supply needed for running the powerful magnet needed to split the absorption line.

Background correction techniques in HR-CS AAS

In HR-CS AAS background correction is carried out mathematically in the software using information from detector pixels that are not used for measuring atomic absorption; hence, in contrast to LS AAS, no additional components are required for background correction.

Background correction using correction pixels

It has already been mentioned that in HR-CS AAS lamp flicker noise is eliminated using correction pixels. In fact, any increase or decrease in radiation intensity that is observed to the same extent at all pixels chosen for correction is eliminated by the correction algorithm.

This obviously also includes a reduction of the measured intensity due to radiation scattering or molecular absorption, which is corrected in the same way. As measurement of total and background absorption, and correction for the latter, are strictly simultaneous (in contrast to LS AAS), even the fastest changes of background absorption, as they may be observed in ET AAS, do not cause any problem. In addition, as the same algorithm is used for background correction and elimination of lamp noise, the background corrected signals show a much better signal-to-noise ratio compared to the uncorrected signals, which is also in contrast to LS AAS.

Background correction using a least-squares algorithm

The above technique can obviously not correct for a background with fine structure, as in this case the absorbance will be different at each of the correction pixels. In this case HR-CS AAS is offering the possibility to measure correction spectra of the molecule(s) that is (are) responsible for the background and store them in the computer. These spectra are then multiplied with a factor to match the intensity of the sample spectrum and subtracted pixel by pixel and spectrum by spectrum from the sample spectrum using a least-squares algorithm. This might sound complex, but first of all the number of di-atomic molecules that can exist at the temperatures of the atomizers used in AAS is relatively small, and second, the correction is performed by the computer within a few seconds. The same algorithm can actually also be used to correct for direct line overlap of two atomic absorption lines, making HR-CS AAS the only AAS technique that can correct for this kind of spectral interference.

Atomic emission spectroscopy

Atomic emission spectroscopy (**AES**) is a method of chemical analysis that uses the intensity of light emitted from a flame, plasma, arc, or spark at a particular wavelength to determine the quantity of an element in a sample. The wavelength of the atomic spectral line in the emission spectrum gives the identity of the element while the intensity of the emitted light is proportional to the number of atoms of the element. The sample may be excited by various methods.

Flame

- A sample of a material (analyte) is brought into the flame as a gas, sprayed solution, or directly inserted into the flame by use of a small loop of wire, usually platinum. The heat from the flame evaporates the solvent and breaks intramolecular bonds to create free atoms. The thermal energy also excites the atoms into excited electronic states that subsequently emit light when they return to the ground electronic state. Each element emits light at a characteristic wavelength, which is dispersed by a grating or prism and detected in the spectrometer.

A frequent application of the emission measurement with the flame is the regulation of alkali metals for pharmaceutical analytics.

Inductively coupled plasma

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) uses an inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element.

Advantages of ICP-AES are the excellent limit of detection and linear dynamic range, multi-element capability, low chemical interference and a stable and reproducible signal. Disadvantages are spectral interferences (many emission lines), cost and operating expense and the fact that samples typically must be in a liquid solution. Inductively Coupled Plasma (ICP)

source of the emission consists of an induction coil and plasma. An induction coil is a coil of wire that has an alternating current flowing through it. This current induces a magnetic field inside the coil, coupling a great deal of energy to plasma contained in a quartz tube inside the coil. Plasma is a collection of charged particles (cations and electrons) capable, by virtue of their charge, of interacting with a magnetic field.The plasmas used inatomic emissions are formed by ionizing a flowing stream of argon gas. Plasma's high-temperature results from resistive heating as the charged particles move through the gas. Because plasmas operate at much higher temperatures than flames, they provide better atomization and a higher population of excited states. The predominant form of sample matrix in ICP-AES today is a liquid sample: acidified water or solids digested into aqueous forms. Liquid samples are pumped into the nebulizer and sample chamber via a peristaltic pump. Then the samples pass through a nebulizer that creates a fine mist of liquid particles. Larger water droplets condense on the sides of the spray chamber and are removed via the drain, while finer water droplets move with the argon flow and enter the plasma. With plasma emission, it is possible to analyze solid samples directly. These procedures include incorporating electrothermal vaporization, laser and spark ablation, and glow-discharge vaporization.

Spark and arc

Spark or arc atomic emission spectroscopy is used for the analysis of metallic elements in solid samples. For non-conductive materials, the sample is ground with graphite

powder to make it conductive. In traditional arc spectroscopy methods, a sample of the solid was commonly ground up and destroyed during analysis. An electric arc or spark is passed through the sample, heating it to a high temperature to excite the atoms within it. The excited analyte atoms emit light at characteristic wavelengths that can be dispersed with a monochromator and detected. In the past, the spark or arc conditions were typically not well controlled, the analysis for the elements in the sample were qualitative. However, modern spark sources with controlled discharges can be considered quantitative. Both qualitative and quantitative spark analysis are widely used for production quality control in foundry and metal casting facilities.

Infrared spectroscopy

Infrared spectroscopy (**IR spectroscopy** or **vibrational spectroscopy**) is the measurement of the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms. The method or technique of infrared spectroscopy is conducted with an instrument called an **infrared spectrometer** (or spectrophotometer) which produces an **infrared spectrum**. An IR spectrum can be visualized in a graph of infrared light absorbance (or transmittance) on the vertical axis vs. frequency or wavelength on the horizontal axis. Typical units of frequency used in IR spectra are reciprocal centimeters (sometimes called wave numbers), with the symbol cm. Units of IR wavelength are commonly given in micrometers (formerly called "microns"), symbol μm, which are related to wave

numbers in a reciprocal way. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer. Two-dimensional IR is also possible as discussed below.

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 14,000–4,000 cm (0.7–2.5 μm wavelength) can excite overtone or combination modes of molecular vibrations. The mid-infrared, approximately 4,000–400 cm (2.5–25 μm) is generally used to study the fundamental vibrations and associated rotational–vibrational structure. The far-infrared, approximately 400–10 cm (25–1,000 μm) has low energy and may be used for rotational spectroscopy and low frequency vibrations. The region from 2–130 cm, bordering the microwave region, is considered the terahertz region and may probe intermolecular vibrations. The names and classifications of these subregions are conventions, and are only loosely based on the relative molecular or electromagnetic properties.

Theory

- Infrared spectroscopy exploits the fact that molecules absorb frequencies that are characteristic of their structure. These absorptions occur at resonant frequencies, i.e. the frequency of the absorbed radiation matches the vibrational frequency. The energies are affected 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 associated with the normal modes of vibration corresponding to the molecular electronic ground state potential energy surface. The resonant frequencies are also related to the strength of the bond and the mass of the atoms at either end of it. Thus, the frequency of the vibrations are associated with a particular normal mode of motion and a particular bond type.

Number of vibrational modes

In order for a vibrational mode in a sample to be "IR active", it must be associated with changes in the dipole moment. 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 number of atoms, 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_2X_2 group, commonly found in organic compounds and where X can represent any other atom, can vibrate in nine different ways. Six of these vibrations involve only the CH_2 portion: two **stretching** modes (ν): **symmetric** (ν_s) and **antisymmetric** (ν_{as}); and four **bending** modes: **scissoring** (δ), **rocking** (ρ), **wagging** (ω) and **twisting** (τ), as shown below. Structures that do not have the two additional X groups attached have fewer modes because some modes are defined by specific relationships to those other attached groups. For example, in water, the rocking, wagging, and twisting modes do not exist because these types of motions of the H atoms represent simple rotation of the whole molecule rather than vibrations within it. In case of more complex molecules,**out-of-plane** (γ) vibrational modes can be also present.

The simplest and most important or *fundamental* IR bands arise from the excitations of normal modes, the simplest distortions of the molecule, from the ground state with vibrational quantum numberυ = 0 to the first excited state with vibrational quantum number υ = 1. In some cases, overtone bandsare observed. An overtone band arises from the absorption of a photon leading to a direct transition from the ground state to the second excited vibrational state (υ = 2). Such a band appears at approximately twice the energy of the fundamental band for the same normal mode. Some excitations, so-called *combination modes*, involve simultaneous excitation of 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 etc.

Practical IR spectroscopy

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 or collection of bonds, absorption occurs. Examination of the transmitted light reveals how much energy was absorbed at each frequency (or wavelength). This measurement can be achieved by scanning the wavelength range using a monochromator. Alternatively, the entire wavelength range is measured using a Fourier transform instrument and then a transmittance or absorbance spectrum is generated using a dedicated procedure.

This technique is commonly used for analyzing 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.

Sample preparation

Gas samples

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 interest.

A simple glass tube with length of 5 to 10 cm equipped with infrared-transparent windows at the both ends of the tube can be used for concentrations down to several hundred ppm. 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

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

Solid samples can be prepared in a variety of ways. One common method is to crush the sample with an oily mulling agent (usually mineral oil Nujol). A thin film of the mull is applied 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.

Comparing to a reference

It is typical to record spectrum of both the sample and a "reference". This step controls for a number of variables, e.g. infrared detector, which may affect the spectrum. The reference measurement makes it possible to eliminate the instrument influence.

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 figure), can correct for these types of effects to give very accurate results. The Standard addition method can be used to statistically cancel these errors.

Nevertheless, among different absorption based techniques which are used for gaseous species detection, Cavity ring-down spectroscopy (CRDS) can be used as a calibration free method. The fact that CRDS is based on the measurements of photon life-times (and not the laser intensity) makes it needless for any calibration and comparison with a reference

FTIR

Fourier transform infrared (FTIR) spectroscopy is a measurement technique that allows one to record infrared spectra. Infrared light is guided through an interferometer 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, wavenumber). As described above, the sample's spectrum is always compared to a reference.

An alternate method for acquiring spectra is the "dispersive" or "scanning monochromator" method. In this approach, the sample is irradiated sequentially with various single wavelengths. 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.

Infrared microscopy

Various forms of infrared microscopy exist. These include IR versions of sub-diffraction microscopy such as IR NSOM, photothermalmicrospectroscopy, Nano-FTIR and atomic force microscope based infrared spectroscopy (AFM-IR).

Other methods in molecular vibrational spectroscopy

Infrared spectroscopy is not the only method of studying molecular vibrational spectra. Raman spectroscopy involves an inelastic scattering process in which only part of the energy of an incident photon is absorbed by the molecule, and the remaining part is scattered and detected. The energy difference corresponds to absorbed vibrational energy.

The selection rules for infrared and for Raman spectroscopy are different at least for some molecular symmetries, so that the two methods are complementary in that they observe vibrations of different symmetries.

Another method is electron energy loss spectroscopy (EELS), in which the energy absorbed is provided by an inelastically scattered electron rather than a photon. This method is useful for studying vibrations of molecules adsorbed on a solid surface.

Recently, high-resolution EELS (HREELS) has emerged as a technique for performing vibrational spectroscopy in a transmission electron microscope (TEM). In combination with the high spatial resolution of the TEM, unprecedented experiments have been performed, such as nano-scale temperature measurements, mapping of isotopically labeled molecules, mapping of phonon modes in position- and momentum-space, vibrational surface and bulk mode mapping on nanocubes, and investigations of polariton modes in van der Waals crystals. Analysis of vibrational modes that are IR-inactive but appear in Inelastic Neutron Scattering is also possible at high spatial resolution using EELS. Although the

spatial resolution of HREELs is very high, the bands are extremely broad compared to other techniques.

Computational infrared microscopy

By using computer simulations and normal modeanalysis it is possible to calculate theoretical frequencies of molecules.

Absorption bands

Badger's rule

For many kinds of samples, the assignments are known, i.e. which bond deformation(s) are associated with which frequency. In such cases further information can be gleaned about the strength on a bond, relying on the empirical guideline called **Badger's Rule**. Originally published by Richard McLean Badger in 1934, this rule states that the strength of a bond (in terms of force constant) correlates with the bond length. That is, increase in bond strength leads to corresponding bond shortening and vice versa.

Uses and 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 determining the blood alcohol content of a suspected drunk driver.

IR-spectroscopy has been successfully used in analysis and identification of pigments in paintings and other art objects such as illuminated manuscripts.

A useful way of analyzing solid samples without the need for cutting samples uses ATR or attenuated total reflectance 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 also automatically identify the substance 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.

Another important application of Infrared Spectroscopy is in the food industry to measure the concentration of various compounds in different food products

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

Infrared Spectroscopy is also used in gas leak detection devices such as the DP-IR and EyeCGAs. These devices detect hydrocarbon gas leaks in the transportation of natural gas and crude oil.

In February 2014, NASA announced a greatly upgraded database, based on IR spectroscopy, for tracking polycyclic aromatic hydrocarbons (PAHs) in the universe. According to scientists, more than 20% of the carbon in the universe may be associated with PAHs, possible starting materials for the formation of life. PAHs seem to have been formed shortly after the Big Bang, are widespread throughout the universe, and are associated with new stars and exoplanets. Infrared spectroscopy is an important analysis method in the recycling process of household waste plastics, and a convenient stand-off method to sort plastic of different polymers (PET, HDPE, ...).

Recent developments include a miniature IR-spectrometer that's linked to a cloud based database and suitable for personal everyday use, and NIR-spectroscopic chips that can be embedded in smartphones and various gadgets.

Two-dimensional IR

- **Two-dimensional infrared correlation spectroscopy analysis** combines multiple samples of infrared spectra to reveal more complex properties. By extending the spectral information of a perturbed sample, spectral analysis is simplified and resolution is enhanced. The 2D synchronous and 2D asynchronous spectra represent a graphical overview of the spectral changes due to a perturbation (such as a changing concentration or changing temperature) as well as the relationship between the spectral changes at two different wavenumbers.

Nonlinear two-dimensional infrared spectroscopy is the infrared version of correlation spectroscopy. Nonlinear two-dimensional infrared spectroscopy is a technique that has become available with the development of femtosecond infrared laser pulses. In this experiment, first a set of pump pulses is applied to the sample. This is followed by a waiting time during which the system is allowed to relax. The typical waiting time lasts from zero to several picoseconds, and the duration can be controlled with a resolution of tens of femtoseconds. A probe pulse is then applied, resulting in the emission of a signal from the sample. The nonlinear two-dimensional infrared spectrum is a two-dimensional correlation plot of the frequency ω_1 that

was excited by the initial pump pulses and the frequency ω_3 excited by the probe pulse after the waiting time. This allows the observation of coupling between different vibrational modes; because of its extremely fine time resolution, it can be used to monitor molecular dynamics on a picosecond timescale. It is still a largely unexplored technique and is becoming increasingly popular for fundamental research.

As with two-dimensional nuclear magnetic resonance (2DNMR) spectroscopy, this technique spreads the spectrum in two dimensions and allows for the observation of cross peaks that contain information on the coupling between different modes. In contrast to 2DNMR, nonlinear two-dimensional infrared spectroscopy also involves the excitation to overtones. These excitations result in excited state absorption peaks located below the diagonal and cross peaks. In 2DNMR, two distinct techniques, COSY and NOESY, are frequently used. The cross peaks in the first are related to the scalar coupling, while in the latter they are related to the spin transfer between different nuclei.

In nonlinear two-dimensional infrared spectroscopy, analogs have been drawn to these 2DNMR techniques. Nonlinear two-dimensional infrared spectroscopy with zero waiting time corresponds to COSY, and nonlinear two-dimensional infrared spectroscopy with finite waiting time allowing vibrational population transfer corresponds to NOESY. The COSY variant of nonlinear two-dimensional infrared spectroscopy has been used for determination of the secondary structure content of proteins.

Melting-point apparatus

A **melting-point apparatus** is a scientific instrument used to determine the melting point of a substance. Some types of melting-point apparatuses include the Thiele tube, Fisher-Johns apparatus, Gallenkamp (Electronic) melting-point apparatus and automatic melting-point apparatus.

Design

While the outward designs of apparatuses can vary greatly, most apparatuses use a sample loaded into a sealed capillary (*melting-point capillary*), which is then placed in the apparatus. The sample is then heated, either by a heating block or an oil bath, and as the temperature increases, the sample is observed to determine when the phase change from solid to liquid occurs. The operator of the apparatus records the temperature range starting with the initial phase-change temperature and ending with the completed phase-change temperature. The temperature range that is determined can then be averaged to gain the melting point of the sample being examined.

Apparatuses usually have a control panel that allows the starting and final temperatures, as well as the temperature gradient (in units per minute), to be programmed. Some machines have several channels, which permit more than one sample to be tested at a time. The control panel might have buttons that allow the start and end of the melting point range to be recorded.

Thiele tube

- A Thiele tube is a glass instrument that is filled with oil that is heated by using an open flame. The sample is placed in the opening in a capillary tube alongside a mercury thermometer and allowed to be heated by the oil as it circulates through the Thiele tube. By using different oils, different temperature ranges can be reached and used to determine melting points. The Thiele tube may also be used to determine boiling points, by using a liquid sample instead of a solid sample.

Microscope

A **microscope** (from Ancient Greek: μικρός*mikrós* 'small' and σκοπεῖν*skopeîn* 'to look (at); examine, inspect') is a laboratory instrument used to examine objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures using a microscope. Microscopic means being invisible to the eye unless aided by a microscope.

There are many types of microscopes, and they may be grouped in different ways. One way is to describe the method an instrument uses to interact with a sample and produce images, either by sending a beam of light or electrons through a sample in its optical path, by detecting photon emissions from a sample, or by scanning across and a short distance from the surface of a sample using a probe. The most common microscope (and the first to be invented) is the optical

microscope, which uses lenses to refractvisible light that passed through a thinly sectioned sample to produce an observable image. Other major types of microscopes are the fluorescence microscope, electron microscope (both the transmission electron microscope and the scanning electron microscope) and various types of scanning probe microscopes.

History

Although objects resembling lenses date back 4,000 years and there are Greek accounts of the optical properties of water-filled spheres (5th century BC) followed by many centuries of writings on optics, the earliest known use of simple microscopes (magnifying glasses) dates back to the widespread use of lenses in eyeglasses in the 13th century. The earliest known examples of compound microscopes, which combine an objective lens near the specimen with an eyepiece to view a real image, appeared in Europe around 1620. The inventor is unknown, even though many claims have been made over the years. Several revolve around the spectacle-making centers in the Netherlands, including claims it was invented in 1590 by Zacharias Janssen (claim made by his son) or Zacharias' father, Hans Martens, or both, claims it was invented by their neighbor and rival spectacle maker, Hans Lippershey (who applied for the first telescope patent in 1608), and claims it was invented by expatriateCornelisDrebbel, who was noted to have a version in London in 1619.Galileo Galilei (also sometimes cited as compound microscope inventor) seems to have found after 1610 that he could close focus his telescope to view small objects and, after seeing a compound microscope built by Drebbel exhibited in Rome in 1624, built his own

improved version. Giovanni Faber coined the name *microscope* for the compound microscope Galileo submitted to the AccademiadeiLincei in 1625 (Galileo had called it the *occhiolino* 'little eye').

Rise of modern light microscopes

The first detailed account of the microscopic anatomy of organic tissue based on the use of a microscope did not appear until 1644, in GiambattistaOdierna's*L'occhiodellamosca*, or *The Fly's Eye*.

The microscope was still largely a novelty until the 1660s and 1670s when naturalists in Italy, the Netherlands and England began using them to study biology. Italian scientist Marcello Malpighi, called the father of histology by some historians of biology, began his analysis of biological structures with the lungs. The publication in 1665 of Robert Hooke's *Micrographia* had a huge impact, largely because of its impressive illustrations. A significant contribution came from Antonie van Leeuwenhoek who achieved up to 300 times magnification using a simple single lens microscope. He sandwiched a very small glass ball lens between the holes in two metal plates riveted together, and with an adjustable-by-screws needle attached to mount the specimen. Then, Van Leeuwenhoek re-discovered red blood cells (after Jan Swammerdam) and spermatozoa, and helped popularise the use of microscopes to view biological ultrastructure. On 9 October 1676, van Leeuwenhoek reported the discovery of micro-organisms.

The performance of a light microscope depends on the quality and correct use of the condensor lens system to focus light on

the specimen and the objective lens to capture the light from the specimen and form an image. Early instruments were limited until this principle was fully appreciated and developed from the late 19th to very early 20th century, and until electric lamps were available as light sources. In 1893August Köhler developed a key principle of sample illumination, Köhler illumination, which is central to achieving the theoretical limits of resolution for the light microscope. This method of sample illumination produces even lighting and overcomes the limited contrast and resolution imposed by early techniques of sample illumination. Further developments in sample illumination came from the discovery of phase contrast by Frits Zernike in 1953, and differential interference contrast illumination by Georges Nomarski in 1955; both of which allow imaging of unstained, transparent samples.

Electron microscopes

In the early 20th century a significant alternative to the light microscope was developed, an instrument that uses a beam of electrons rather than light to generate an image. The German physicist, Ernst Ruska, working with electrical engineer Max Knoll, developed the first prototype electron microscope in 1931, a transmission electron microscope (TEM). The transmission electron microscope works on similar principles to an optical microscope but uses electrons in the place of light and electromagnets in the place of glass lenses. Use of electrons, instead of light, allows for much higher resolution.

Development of the transmission electron microscope was quickly followed in 1935 by the development of the scanning electron microscope by Max Knoll. Although TEMs were being

used for research before WWII, and became popular afterwards, the SEM was not commercially available until 1965.

Transmission electron microscopes became popular following the Second World War. Ernst Ruska, working at Siemens, developed the first commercial transmission electron microscope and, in the 1950s, major scientific conferences on electron microscopy started being held. In 1965, the first commercial scanning electron microscope was developed by Professor Sir Charles Oatley and his postgraduate student Gary Stewart, and marketed by the Cambridge Instrument Company as the "Stereoscan".

One of the latest discoveries made about using an electron microscope is the ability to identify a virus. Since this microscope produces a visible, clear image of small organelles, in an electron microscope there is no need for reagents to see the virus or harmful cells, resulting in a more efficient way to detect pathogens.

Scanning probe microscopes

From 1981 to 1983Gerd Binnig and Heinrich Rohrer worked at IBM in Zurich, Switzerland to study the quantum tunnelling phenomenon. They created a practical instrument, a scanning probe microscope from quantum tunnellingtheory, that read very small forces exchanged between a probe and the surface of a sample. The probe approaches the surface so closely that electrons can flow continuously between probe and sample, making a current from surface to probe. The microscope was not initially well received due to the complex nature of the underlying theoretical explanations. In 1984 Jerry Tersoff and

D.R. Hamann, while at AT&T's Bell Laboratories in Murray Hill, New Jersey began publishing articles that tied theory to the experimental results obtained by the instrument. This was closely followed in 1985 with functioning commercial instruments, and in 1986 with Gerd Binnig, Quate, and Gerber's invention of the atomic force microscope, then Binnig's and Rohrer's Nobel Prize in Physics for the SPM.

New types of scanning probe microscope have continued to be developed as the ability to machine ultra-fine probes and tips has advanced.

Fluorescence microscopes

The most recent developments in light microscope largely centre on the rise of fluorescence microscopy in biology. During the last decades of the 20th century, particularly in the post-genomic era, many techniques for fluorescent staining of cellular structures were developed. The main groups of techniques involve targeted chemical staining of particular cell structures, for example, the chemical compound DAPI to label DNA, use of antibodies conjugated to fluorescent reporters, see immunofluorescence, and fluorescent proteins, such as green fluorescent protein. These techniques use these different fluorophores for analysis of cell structure at a molecular level in both live and fixed samples.

The rise of fluorescence microscopy drove the development of a major modern microscope design, the confocal microscope. The principle was patented in 1957 by Marvin Minsky, although laser technology limited practical application of the technique. It was not until 1978 when Thomas and Christoph Cremer

developed the first practical confocal laser scanning microscope and the technique rapidly gained popularity through the 1980s.

Super resolution microscopes

Much current research (in the early 21st century) on optical microscope techniques is focused on development of superresolution analysis of fluorescently labelled samples. Structured illumination can improve resolution by around two to four times and techniques like stimulated emission depletion (STED) microscopy are approaching the resolution of electron microscopes. This occurs because the diffraction limit is occurred from light or excitation, which makes the resolution must be doubled to become super saturated. Stefan Hell was awarded the 2014 Nobel Prize in Chemistry for the development of the STED technique, along with Eric Betzig and William Moerner who adapted fluorescence microscopy for single-molecule visualization.

X-ray microscopes

X-ray microscopes are instruments that use electromagnetic radiation usually in the soft X-ray band to image objects. Technological advances in X-ray lens optics in the early 1970s made the instrument a viable imaging choice. They are often used in tomography (see micro-computed tomography) to produce three dimensional images of objects, including biological materials that have not been chemically fixed. Currently research is being done to improve optics for hard X-rays which have greater penetrating power.

Types

Microscopes can be separated into several different classes. One grouping is based on what interacts with the sample to generate the image, i.e., light or photons (optical microscopes), electrons (electron microscopes) or a probe (scanning probe microscopes). Alternatively, microscopes can be classified based on whether they analyze the sample via a scanning point (confocal optical microscopes, scanning electron microscopes and scanning probe microscopes) or analyze the sample all at once (wide field optical microscopes and transmission electron microscopes).

Wide field optical microscopes and transmission electron microscopes both use the theory of lenses (optics for light microscopes and electromagnet lenses for electron microscopes) in order to magnify the image generated by the passage of a wave transmitted through the sample, or reflected by the sample. The waves used are electromagnetic (in optical microscopes) or electron beams (in electron microscopes). Resolution in these microscopes is limited by the wavelength of the radiation used to image the sample, where shorter wavelengths allow for a higher resolution.

Scanning optical and electron microscopes, like the confocal microscope and scanning electron microscope, use lenses to focus a spot of light or electrons onto the sample then analyze the signals generated by the beam interacting with the sample. The point is then scanned over the sample to analyze a rectangular region. Magnification of the image is achieved by displaying the data from scanning a physically small sample area on a relatively large screen. These microscopes have the

same resolution limit as wide field optical, probe, and electron microscopes. Scanning probe microscopes also analyze a single point in the sample and then scan the probe over a rectangular sample region to build up an image. As these microscopes do not use electromagnetic or electron radiation for imaging they are not subject to the same resolution limit as the optical and electron microscopes described above.

Optical

The most common type of microscope (and the first invented) is the optical microscope. This is an opticalinstrument containing one or more lenses producing an enlarged image of a sample placed in the focal plane. Optical microscopes have refractive glass (occasionally plastic or quartz), to focus light on the eye or on to another light detector. Mirror-based optical microscopes operate in the same manner. Typical magnification of a light microscope, assuming visible range light, is up to 1250x with a theoretical resolution limit of around 0.250 micrometres or 250 nanometres. This limits practical magnification to ~1500x. Specialized techniques (e.g., scanning confocal microscopy, Vertico SMI) may exceed this magnification but the resolution is diffraction limited. The use of shorter wavelengths of light, such as ultraviolet, is one way to improve the spatial resolution of the optical microscope, as are devices such as the near-field scanning optical microscope.

Sarfus is a recent optical technique that increases the sensitivity of a standard optical microscope to a point where it is possible to directly visualize nanometric films (down to 0.3 nanometre) and isolated nano-objects (down to 2 nm-

diameter). The technique is based on the use of non-reflecting substrates for cross-polarized reflected light microscopy.

Ultraviolet light enables the resolution of microscopic features as well as the imaging of samples that are transparent to the eye. Near infrared light can be used to visualize circuitry embedded in bonded silicon devices, since silicon is transparent in this region of wavelengths.

In fluorescence microscopy many wavelengths of light ranging from the ultraviolet to the visible can be used to cause samples to fluoresce, which allows viewing by eye or with specifically sensitive cameras.

Phase-contrast microscopy is an optical microscopy illumination technique in which small phase shifts in the light passing through a transparent specimen are converted into amplitude or contrast changes in the image. The use of phase contrast does not require staining to view the slide. This microscope technique made it possible to study the cell cycle in live cells.

The traditional optical microscope has more recently evolved into the digital microscope. In addition to, or instead of, directly viewing the object through the eyepieces, a type of sensor similar to those used in a digital camerais used to obtain an image, which is then displayed on a computer monitor. These sensors may use CMOS or charge-coupled device (CCD) technology, depending on the application.

Digital microscopy with very low light levels to avoid damage to vulnerable biological samples is available using sensitive photon-counting digital cameras. It has been demonstrated

that a light source providing pairs of entangled photons may minimize the risk of damage to the most light-sensitive samples. In this application of ghost imaging to photon-sparse microscopy, the sample is illuminated with infrared photons, each of which is spatially correlated with an entangled partner in the visible band for efficient imaging by a photon-counting camera.

Electron

The two major types of electron microscopes are transmission electron microscopes (TEMs) and scanning electron microscopes (SEMs). They both have series of electromagnetic and electrostatic lenses to focus a high energy beam of electrons on a sample. In a TEM the electrons pass through the sample, analogous to basic optical microscopy. This requires careful sample preparation, since electrons are scattered strongly by most materials. The samples must also be very thin (below 100 nm) in order for the electrons to pass through it. Cross-sections of cells stained with osmium and heavy metals reveal clear organelle membranes and proteins such as ribosomes. With a 0.1 nm level of resolution, detailed views of viruses (20 – 300 nm) and a strand of DNA (2 nm in width) can be obtained. In contrast, the SEM has raster coils to scan the surface of bulk objects with a fine electron beam. Therefore, the specimen do not necessarily need to be sectioned, but coating with a nanometric metal or carbon layer may be needed for nonconductive samples. SEM allows fast surface imaging of samples, possibly in thin water vapor to prevent drying.

Scanning probe

- The different types of scanning probe microscopes arise from the many different types of interactions that occur when a small probe is scanned over and interacts with a specimen. These interactions or modes can be recorded or mapped as function of location on the surface to form a characterization map. The three most common types of scanning probe microscopes are atomic force microscopes (AFM), near-field scanning optical microscopes (MSOM or SNOM, scanning near-field optical microscopy), and scanning tunneling microscopes (STM). An atomic force microscope has a fine probe, usually of silicon or silicon nitride, attached to a cantilever; the probe is scanned over the surface of the sample, and the forces that cause an interaction between the probe and the surface of the sample are measured and mapped. A near-field scanning optical microscope is similar to an AFM but its probe consists of a light source in an optical fiber covered with a tip that has usually an aperture for the light to pass through. The microscope can capture either transmitted or reflected light to measure very localized optical properties of the surface, commonly of a biological specimen. Scanning tunneling microscopes have a metal tip with a single apical atom; the tip is attached to a tube through which a current flows. The tip is scanned over the surface of a conductive sample until a tunneling current flows; the current is kept constant by computer movement

of the tip and an image is formed by the recorded movements of the tip.

Other types

Scanning acoustic microscopes use sound waves to measure variations in acoustic impedance. Similar to Sonar in principle, they are used for such jobs as detecting defects in the subsurfaces of materials including those found in integrated circuits. On February 4, 2013, Australian engineers built a "quantum microscope" which provides unparalleled precision.

Optical spectrometer

An **optical spectrometer** (**spectrophotometer**, **spectrograph** or **spectroscope**) is an instrument used to measure properties of light over a specific portion of the electromagnetic spectrum, typically used in spectroscopic analysis to identify materials. The variable measured is most often the light's intensity but could also, for instance, be the polarization state. The independent variable is usually the wavelength of the light or a unit directly proportional to the photon energy, such as reciprocal centimeters or electron volts, which has a reciprocal relationship to wavelength.

A spectrometeris used in spectroscopy for producing spectral lines and measuring their wavelengths and intensities. Spectrometers may operate over a wide range of non-optical wavelengths, from gamma rays and X-rays into the far infrared. If the instrument is designed to measure the spectrum on an absolute scale rather than a relative one, then it is typically called a spectrophotometer. The majority of

spectrophotometers are used in spectral regions near the visible spectrum.

In general, any particular instrument will operate over a small portion of this total range because of the different techniques used to measure different portions of the spectrum. Below optical frequencies (that is, at microwave and radio frequencies), the spectrum analyzer is a closely related electronic device.

Spectrometers are used in many fields. For example, they are used in astronomy to analyze the radiation from objects and deduce their chemical composition. The spectrometer uses a prism or a grating to spread the light into a spectrum. This allows astronomers to detect many of the chemical elements by their characteristic spectral lines. These lines are named for the elements which cause them, such as the hydrogen alpha, beta, and gamma lines. A glowing object will show bright spectral lines. Dark lines are made by absorption, for example by light passing through a gas cloud, and these absorption lines can also identify chemical compounds. Much of our knowledge of the chemical makeup of the universe comes from spectra.

Spectroscopes

Spectroscopes are often used in astronomy and some branches of chemistry. Early spectroscopes were simply prisms with graduations marking wavelengths of light. Modern spectroscopes generally use a diffraction grating, a movable slit, and some kind of photodetector, all automated and controlled by a computer.

Joseph von Fraunhofer developed the first modern spectroscope by combining a prism, diffraction slit and telescope in a manner that increased the spectral resolution and was reproducible in other laboratories. Fraunhofer also went on to invent the first diffraction spectroscope. Gustav Robert Kirchhoff and Robert Bunsen discovered the application of spectroscopes to chemical analysis and used this approach to discover caesium and rubidium. Kirchhoff and Bunsen's analysis also enabled a chemical explanation of stellar spectra, including Fraunhofer lines.

When a material is heated to incandescence it emits light that is characteristic of the atomic makeup of the material. Particular light frequencies give rise to sharply defined bands on the scale which can be thought of as fingerprints. For example, the element sodium has a very characteristic double yellow band known as the Sodium D-lines at 588.9950 and 589.5924 nanometers, the color of which will be familiar to anyone who has seen a low pressuresodium vapor lamp.

In the original spectroscope design in the early 19th century, light entered a slit and a collimating lens transformed the light into a thin beam of parallel rays. The light then passed through a prism (in hand-held spectroscopes, usually an Amici prism) that refracted the beam into a spectrum because different wavelengths were refracted different amounts due to dispersion. This image was then viewed through a tube with a scale that was transposed upon the spectral image, enabling its direct measurement.

With the development of photographic film, the more accurate spectrographwas created. It was based on the same principle

as the spectroscope, but it had a camera in place of the viewing tube. In recent years, the electronic circuits built around the photomultiplier tube have replaced the camera, allowing real-time spectrographic analysis with far greater accuracy. Arrays of photosensorsare also used in place of film in spectrographic systems. Such spectral analysis, or spectroscopy, has become an important scientific tool for analyzing the composition of unknown material and for studying astronomical phenomena and testing astronomical theories.

In modern spectrographs in the UV, visible, and near-IR spectral ranges, the spectrum is generally given in the form of photon number per unit wavelength (nm or μm), wavenumber (μm, cm), frequency (THz), or energy (eV), with the units indicated by the abscissa. In the mid- to far-IR, spectra are typically expressed in units of Watts per unit wavelength (μm) or wavenumber (cm). In many cases, the spectrum is displayed with the units left implied (such as "digital counts" per spectral channel).

Spectrographs

A spectrograph is an instrument that separates light by its wavelengths and records this data. A spectrograph typically has a multi-channel detector system or camera that detects and records the spectrum of light.

The term was first used in 1876 by Dr. Henry Draper when he invented the earliest version of this device, and which he used to take several photographs of the spectrum of Vega. This

earliest version of the spectrograph was cumbersome to use and difficult to manage.

There are several kinds of machines referred to as *spectrographs*, depending on the precise nature of the waves. The first spectrographs used photographic paper as the detector. The plant pigment phytochromewas discovered using a spectrograph that used living plants as the detector. More recent spectrographs use electronic detectors, such as CCDs which can be used for both visible and UV light. The exact choice of detector depends on the wavelengths of light to be recorded.

A spectrograph is sometimes calledpolychromator, as an analogy to monochromator.

Stellar and solar spectrograph

The star spectral classification and discovery of the main sequence, Hubble's law and the Hubble sequencewere all made with spectrographs that used photographic paper. The forthcoming James Webb Space Telescope will contain both a near-infrared spectrograph (NIRSpec) and a mid-infrared spectrograph (MIRI).

Echelle spectrograph

An Echelle spectrograph uses two diffraction gratings, rotated 90 degrees with respect to each other and placed close to one another. Therefore, an entrance point and not a slit is used and a 2d CCD-chip records the spectrum. Usually one would guess to retrieve a spectrum on the diagonal, but when both gratings have a wide spacing and one is blazed so that only the

first order is visible and the other is blazed that a lot of higher orders are visible, one gets a very fine spectrum nicely folded onto a small common CCD-chip. The small chip also means that the collimating optics need not to be optimized for coma or astigmatism, but the spherical aberration can be set to zero.

Spectrophotometry

Spectrophotometry is a branch of electromagnetic spectroscopy concerned with the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. Spectrophotometry uses photometers, known as spectrophotometers, that can measure the intensity of a light beam at different wavelengths. Although spectrophotometry is most commonly applied to ultraviolet, visible, and infrared radiation, modern spectrophotometers can interrogate wide swaths of the electromagnetic spectrum, including x-ray, ultraviolet, visible, infrared, and/or microwave wavelengths.

Overview

Spectrophotometry is a tool that hinges on the quantitative analysis of molecules depending on how much light is absorbed by colored compounds. Important features of spectrophotometers are spectral bandwidth (the range of colors it can transmit through the test sample), the percentage of sample-transmission, the logarithmic range of sample-absorption, and sometimes a percentage of reflectance measurement.

A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. Although many biochemicals are colored, as in, they absorb visible light and therefore can be measured by colorimetric procedures, even colorless biochemicals can often be converted to colored compounds suitable for chromogenic color-forming reactions to yield compounds suitable for colorimetric analysis. However, they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm - 2500 nm using different controls and calibrations. Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the *photometric determination*.

An example of an experiment in which spectrophotometry is used is the determination of the equilibrium constant of a solution. A certain chemical reaction within a solution may occur in a forward and reverse direction, where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of reactants and products at this point, the light transmittance of the solution can be tested using spectrophotometry. The amount of light that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

The absorption of light is due to the interaction of light with the electronic and vibrational modes of molecules. Each type of molecule has an individual set of energy levels associated with the makeup of its chemical bonds and nuclei and thus will

absorb light of specific wavelengths, or energies, resulting in unique spectral properties. This is based upon its specific and distinct makeup.

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, Chemical Engineering, and molecular biology. They are widely used in many industries including semiconductors, laser and optical manufacturing, printing and forensic examination, as well in laboratories for the study of chemical substances. Spectrophotometry is often used in measurements of enzyme activities, determinations of protein concentrations, determinations of enzymatic kinetic constants, and measurements of ligand binding reactions. Ultimately, a spectrophotometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths.

In astronomy, the term spectrophotometry refers to the measurement of the spectrum of a celestial object in which the flux scale of the spectrum is calibrated as a function of wavelength, usually by comparison with an observation of a spectrophotometric standard star, and corrected for the absorption of light by the Earth's atmosphere.

History

Invented by Arnold O. Beckman in 1940, the spectrophotometer was created with the aid of his colleagues at his company National Technical Laboratories founded in 1935 which would become Beckman Instrument Company and

ultimately Beckman Coulter. This would come as a solution to the previously created spectrophotometers which were unable to absorb the ultraviolet correctly. He would start with the invention of Model A where a glass prism was used to absorb the UV light. It would be found that this did not give satisfactory results, therefore in Model B, there was a shift from a glass to a quartz prism which allowed for better absorbance results. From there, Model C was born with an adjustment to the wavelength resolution which ended up having three units of it produced. The last and most popular model became Model D which is better recognized now as the DU spectrophotometer which contained the instrument case, hydrogen lamp with ultraviolent continuum and a better monochromator. It was produced from 1941 to 1976 where the price for it in 1941 was US$723 (far-UV accessories were an option at additional cost). In the words of Nobel chemistry laureate Bruce Merrifield, it was "probably the most important instrument ever developed towards the advancement of bioscience."

Once it became discontinued in 1976, Hewlett-Packard created the first commercially available diode-array spectrophotometer in 1979 known as the HP 8450A. Diode-array spectrophotometers differed from the original spectrophotometer created by Beckman because it was the first single-beam microprocessor-controlled spectrophotometer that scanned multiple wavelengths at a time in seconds. It irradiates the sample with polychromatic light which the sample absorbs depending on its properties. Then it is transmitted back by grating the photodiode array which detects the wavelength region of the spectrum. Since then, the creation and implementation of spectrophotometry devices has

increased immensely and has become one of the most innovative instruments of our time.

Design

There are two major classes of devices: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single-beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted. Although comparison measurements from double-beam instruments are easier and more stable, single-beam instruments can have a larger dynamic range and are optically simpler and more compact. Additionally, some specialized instruments, such as spectrophotometers built onto microscopes or telescopes, are single-beam instruments due to practicality.

Historically, spectrophotometers use a monochromator containing a diffraction grating to produce the analytical spectrum. The grating can either be movable or fixed. If a single detector, such as a photomultiplier tube or photodiode is used, the grating can be scanned stepwise (scanning spectrophotometer) so that the detector can measure the light intensity at each wavelength (which will correspond to each "step"). Arrays of detectors (array spectrophotometer), such as charge coupled devices (CCD) or photodiode arrays (PDA) can also be used. In such systems, the grating is fixed and the intensity of each wavelength of light is measured by a different detector in the array. Additionally, most modern mid-infrared spectrophotometers use a Fourier transform technique to

acquire the spectral information. This technique is calledFourier transform infrared spectroscopy.

When making transmission measurements, the spectrophotometer quantitatively compares the fraction of light that passes through a reference solution and a test solution, then electronically compares the intensities of the two signals and computes the percentage of transmission of the sample compared to the reference standard. For reflectance measurements, the spectrophotometer quantitatively compares the fraction of light that reflects from the reference and test samples. Light from the source lamp is passed through a monochromator, which diffracts the light into a "rainbow" of wavelengths through a rotating prism and outputs narrow bandwidths of this diffracted spectrum through a mechanical slit on the output side of the monochromator. These bandwidths are transmitted through the test sample. Then the photon flux density (watts per metre squared usually) of the transmitted or reflected light is measured with a photodiode, charge coupled device or other light sensor. The transmittance or reflectance value for each wavelength of the test sample is then compared with the transmission or reflectance values from the reference sample. Most instruments will apply a logarithmic function to the linear transmittance ratio to calculate the 'absorbency' of the sample, a value which is proportional to the 'concentration' of the chemical being measured.

In short, the sequence of events in a scanning spectrophotometer is as follows:

- The light source is shone into a monochromator, diffracted into a rainbow, and split into two beams. It is then scanned through the sample and the reference solutions.
- Fractions of the incident wavelengths are transmitted through, or reflected from, the sample and the reference.
- The resultant light strikes the photodetector device, which compares the relative intensity of the two beams.
- Electronic circuits convert the relative currents into linear transmission percentages and/or absorbance/concentration values.

In an array spectrophotometer, the sequence is as follows:

- The light source is shone into the sample and focused into a slit
- The transmitted light is refracted into a rainbow with the reflection grating
- The resulting light strikes the photodetector device which compares the intensity of the beam
- Electronic circuits convert the relative currents into linear transmission percentages and/or absorbance/concentration values

Many older spectrophotometers must be calibrated by a procedure known as "zeroing", to balance the null current output of the two beams at the detector. The transmission of a reference substance is set as a baseline (datum) value, so the transmission of all other substances are recorded relative to the initial "zeroed" substance. The spectrophotometer then

converts the transmission ratio into 'absorbency', the concentration of specific components of the test sample relative to the initial substance.

Applications in biochemistry

Spectrophotometry is an important technique used in many biochemical experiments that involve DNA, RNA, and protein isolation, enzyme kinetics and biochemical analyses. Since samples in these applications are not readily available in large quantities, they are especially suited to being analyzed in this non-destructive technique. In addition, precious sample can be saved by utilizing a micro-volume platform where as little as 1uL of sample is required for complete analyses. A brief explanation of the procedure of spectrophotometry includes comparing the absorbency of a blank sample that does not contain a colored compound to a sample that contains a colored compound. This coloring can be accomplished by either a dye such as Coomasie Brilliant Blue G-250 dye measured at 595 nm or by an enzymatic reaction as seen between β-galactosidase and ONPG (turns sample yellow) measured at 420 nm. The spectrophotometer is used to measure colored compounds in the visible region of light (between 350 nm and 800 nm), thus it can be used to find more information about the substance being studied. In biochemical experiments, a chemical and/or physical property is chosen and the procedure that is used is specific to that property in order to derive more information about the sample, such as the quantity, purity, enzyme activity, etc. Spectrophotometry can be used for a number of techniques such as determining optimal wavelength absorbance of samples, determining optimal pH for absorbance of samples, determining concentrations of unknown samples,

and determining the pKa of various samples. Spectrophotometry is also a helpful process for protein purification and can also be used as a method to create optical assays of a compound. Spectrophotometric data can also be

used in conjunction with the Beer–Lambert Equation, , in order to determine various relationships between transmittance and concentration, and absorbance and concentration. Because a spectrophotometer measures the wavelength of a compound through its color, a dye binding substance can be added so that it can undergo a color change and be measured. It is possible to know the concentrations of a two component mixture using the absorption spectra of the standard solutions of each component. To do this, it is necessary to know the extinction coefficient of this mixture at two wave lengths and the extinction coefficients of solutions that contain the known weights of the two components. Spectrophotometers have been developed and improved over decades and have been widely used among chemists. Additionally, Spectrophotometers are specialized to measure either UV or Visible light wavelength absorbance values. It is considered to be a highly accurate instrument that is also very sensitive and therefore extremely precise, especially in determining color change. This method is also convenient for use in laboratory experiments because it is an inexpensive and relatively simple process.

UV-visible spectrophotometry

Most spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near-infrared region as well. The

concentration of a protein can be estimated by measuring the OD at 280 nm due to the presence of tryptophan, tyrosine and phenylalanine. This method is not very accurate since the composition of proteins varies greatly and proteins with none of these amino acids do not have maximum absorption at 280 nm. Nucleic acid contamination can also interfere. This method requires a spectrophotometer capable of measuring in the UV region with quartz cuvettes.

Ultraviolet-visible (UV-vis) spectroscopy involves energy levels that excite electronic transitions. Absorption of UV-vis light excites molecules that are in ground-states to their excited-states.

Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science. It is a known fact that it operates best at the range of 0.2–0.8 O.D. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They take readings in the region of every 5–20 nanometers along the visible region, and produce a spectral reflectance curve or a data stream for alternative presentations. These curves can be used to test a new batch of colorant to check if it makes a match to specifications, e.g., ISO printing standards.

Traditional visible region spectrophotometers cannot detect if a colorant or the base material has fluorescence. This can make it difficult to manage color issues if for example one or more of the printing inks is fluorescent. Where a colorant contains fluorescence, a bi-spectral fluorescent spectrophotometer is used. There are two major setups for visual spectrum spectrophotometers, d/8 (spherical) and 0/45. The names are

due to the geometry of the light source, observer and interior of the measurement chamber. Scientists use this instrument to measure the amount of compounds in a sample. If the compound is more concentrated more light will be absorbed by the sample; within small ranges, the Beer–Lambert law holds and the absorbance between samples vary with concentration linearly. In the case of printing measurements two alternative settings are commonly used- without/with uv filter to control better the effect of uv brighteners within the paper stock.

Samples are usually prepared in cuvettes; depending on the region of interest, they may be constructed of glass, plastic (visible spectrum region of interest), or quartz (Far UV spectrum region of interest). Some applications require small volume measurements which can be performed with micro-volume platforms.

Applications

- Estimating dissolved organic carbon concentration
- Specific ultraviolet absorbance for metric of aromaticity
- Bial's test for concentration of pentoses

Experimental application

As described in the applications section, spectrophotometry can be used in both qualitative and quantitative analysis of DNA, RNA, and proteins. Qualitative analysis can be used and spectrophotometers are used to record spectra of compounds by scanning broad wavelength regions to determine the absorbance properties (the intensity of the color) of the

compound at each wavelength. One experiment that can demonstrate the various uses that visible spectrophotometry can have is the separation of β-galactosidase from a mixture of various proteins. Largely, spectrophotometry is best used to help quantify the amount of purification your sample has undergone relative to total protein concentration. By running an affinity chromatography, B-Galactosidase can be isolated and tested by reacting collected samples with ONPG and determining if the sample turns yellow. Following this testing the sample at 420 nm for specific interaction with ONPG and at 595 for a Bradford Assay the amount of purification can be assessed quantitatively. In addition to this spectrophotometry can be used in tandem with other techniques such as SDS-Page electrophoresis in order to purify and isolate various protein samples.

IR spectrophotometry

Spectrophotometers designed for the infrared region are quite different because of the technical requirements of measurement in that region. One major factor is the type of photosensors that are available for different spectral regions, but infrared measurement is also challenging because virtually everything emits IR light as thermal radiation, especially at wavelengths beyond about 5 μm.

Another complication is that quite a few materials such as glass and plastic absorb infrared light, making it incompatible as an optical medium. Ideal optical materials are salts, which do not absorb strongly. Samples for IR spectrophotometry may be smeared between two discs of potassium bromide or ground

with potassium bromide and pressed into a pellet. Where aqueous solutions are to be measured, insoluble silver chloride is used to construct the cell.

Spectroradiometers

Spectroradiometers, which operate almost like the visible region spectrophotometers, are designed to measure the spectral density of illuminants. Applications may include evaluation and categorization of lighting for sales by the manufacturer, or for the customers to confirm the lamp they decided to purchase is within their specifications. Components:

- The light source shines onto or through the sample.
- The sample transmits or reflects light.
- The detector detects how much light was reflected from or transmitted through the sample.
- The detector then converts how much light the sample transmitted or reflected into a number.

Chapter 3

Techniques

Calorimetry

Calorimetry is the science or act of measuring changes in *state variables* of a body for the purpose of deriving the heat transfer associated with changes of its state due, for example, to chemical reactions, physical changes, or phase transitions under specified constraints. Calorimetry is performed with a calorimeter. The word *calorimetry*is derived from the Latin word *calor*, meaning heat and the Greek word *μέτρον* (metron), meaning measure. 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 the science of calorimetry.

Indirect calorimetry calculates heat that living organisms produce by measuring either their production of carbon dioxide and nitrogen waste (frequently ammonia in aquatic organisms, or urea in terrestrial ones), or from their consumption of oxygen. Lavoisier noted in 1780 that heat production can be predicted from oxygen consumption this way, using multiple regression. The dynamic energy budget theory explains why this procedure is correct. Heat generated by living organisms may also be measured by *direct calorimetry*, in which the entire organism is placed inside the calorimeter for the measurement.

A widely used modern instrument is the differential scanning calorimeter, a device which allows thermal data to be obtained

on small amounts of material. It involves heating the sample at a controlled rate and recording the heat flow either into or from the specimen.

Classical calorimetric calculation of heat

Cases with differentiable equation of state for a one-component body

Basic classical calculation with respect to volume

- Calorimetry requires that a reference material that changes temperature have known definite thermal constitutive properties. The classical rule, recognized by Clausius and Kelvin, is that the pressure exerted by the calorimetric material is fully and rapidly determined solely by its temperature and volume; this rule is for changes that do not involve phase change, such as melting of ice. There are many materials that do not comply with this rule, and for them, the present formula of classical calorimetry does not provide an adequate account. Here the classical rule is assumed to hold for the calorimetric material being used, and the propositions are mathematically written:

Calorimetry through phase change, equation of state shows one jump discontinuity

- An early calorimeter was that used by Laplace and Lavoisier, as shown in the figure above. It worked at constant temperature, and at atmospheric pressure. The latent heat involved was then not a latent heat with respect to volume or with respect to pressure, as in the above account for calorimetry without phase change. The latent heat involved in this calorimeter was with respect to phase change, naturally occurring at constant temperature. This kind of calorimeter worked by measurement of mass of water produced by the melting of ice, which is a phase change.

Physical scope of the above rules of calorimetry

The above rules refer only to suitable calorimetric materials. The terms 'rapidly' and 'very small' call for empirical physical checking of the domain of validity of the above rules.

The above rules for the calculation of heat belong to pure calorimetry. They make no reference to thermodynamics, and were mostly understood before the advent of thermodynamics. They are the basis of the 'thermo' contribution to thermodynamics. The 'dynamics' contribution is based on the idea of work, which is not used in the above rules of calculation.

Electroanalytical methods

Electroanalytical methods are a class of techniques in analytical chemistry which study an analyte by measuring the potential (volts) and/or current (amperes) in an electrochemical cell containing the analyte. These methods can be broken down into several categories depending on which aspects of the cell are controlled and which are measured. The three main categories are potentiometry (the difference in electrode potentials is measured), coulometry (the cell's current is measured over time), and voltammetry (the cell's current is measured while actively altering the cell's potential).

Potentiometry

Potentiometry passively measures the potential of a solution between two electrodes, affecting the solution very little in the process. One electrode is called the reference electrode and has a constant potential, while the other one is an indicator electrode whose potential changes with the composition of the sample. Therefore, the difference of potential between the two electrodes gives an assessment of the composition of the sample. In fact, since potentiometric measurement is a non-destructive measurement, assuming that the electrode is in equilibrium with the solution we are measuring the potential of the solution. Potentiometry usually uses indicator electrodes made *selectively* sensitive to the ion of interest, such as fluoride in fluoride selective electrodes, so that the potential solely depends on the activity of this ion of interest. The time that takes the electrode to establish equilibrium with the solution will affect the sensitivity or accuracy of the

measurement. In aquatic environments, platinum is often used due to its high electron transfer kinetics, although an electrode made from several metals can be used in order to enhance the electron transfer kinetics. The most common potentiometric electrode is by far the glass-membrane electrode used in a pH meter.

A variant of potentiometry is chronopotentiometry which consists in using a constant current and measurement of potential as a function of time. It has been initiated by Weber.

Coulometry

Coulometry uses applied current or potential to completely convert an analyte from one oxidation state to another. In these experiments, the total current passed is measured directly or indirectly to determine the number of electrons passed. Knowing the number of electrons passed can indicate the concentration of the analyte or, when the concentration is known, the number of electrons transferred in the redox reaction. Common forms of coulometry include bulk electrolysis, also known as *Potentiostaticcoulometry* or *controlled potential coulometry*, as well as a variety of coulometric titrations.

Voltammetry

Voltammetry applies a constant and/or varying potential at an electrode's surface and measures the resulting current with a three electrode system. This method can reveal the reduction potential of an analyte and its electrochemical reactivity. This

method in practical terms is nondestructive since only a very small amount of the analyte is consumed at the two-dimensional surface of the working and auxiliary electrodes. In practice the analyte solutions is usually disposed of since it is difficult to separate the analyte from the bulk electrolyte and the experiment requires a small amount of analyte. A normal experiment may involve 1–10 mL solution with an analyte concentration between 1 and 10 mmol/L. Chemically modified electrodes are employed for analysis of organic and inorganic samples.

Polarography

Polarography is a subclass of voltammetry that uses a dropping mercury electrode as the working electrode.

Amperometry

Amperometry is the term indicating the whole of electrochemical techniques in which a current is measured as a function of an independent variable that is, typically, time or electrode potential. Chronoamperometry is the technique in which the current is measured, at a fixed potential, at different times since the start of polarisation. Chronoamperometry is typically carried out in unstirred solution and at fixed electrode, i.e., under experimental conditions avoiding convection as the mass transfer to the electrode. On the other hand, voltammetry is a subclass of amperometry, in which the current is measured by varying the potential applied to the electrode. According to the waveform that describes the way how the potential is varied as a function of time, the different voltammetric techniques are defined.

Gravimetric analysis

Gravimetric analysis describes a set of methods used in analytical chemistry for the quantitative determination of an analyte (the ion being analyzed) based on its mass. The principle of this type of analysis is that once an ion's mass has been determined as a unique compound, that known measurement can then be used to determine the same analyte's mass in a mixture, as long as the relative quantities of the other constituents are known.

The four main types of this method of analysis are *precipitation*, *volatilization*, *electro-analytical* and *miscellaneous physical method*. The methods involve changing the phase of the analyte to separate it in its pure form from the original mixture and are quantitative measurements.

Precipitation method

The precipitation method is the one used for the determination of the amount of calcium in water. Using this method, an excess of oxalic acid, $H_2C_2O_4$, is added to a measured, known volume of water. By adding a reagent, here ammonium oxalate, the calcium will precipitate as calcium oxalate. The proper reagent, when added to aqueous solution, will produce highly insoluble precipitates from the positive and negative ions that would otherwise be soluble with their counterparts (equation 1).

The reaction is:

Formation of calcium oxalate:

$Ca_{(aq)} + C_2O_4 \rightarrow CaC_2O_4$

The precipitate is collected, dried and ignited to high (red) heat which converts it entirely to calcium oxide.

The reaction is pure calcium oxide formed

$CaC_2O_4 \rightarrow CaO_{(s)} + CO_{(g)} + CO_{2(g)}$

The pure precipitate is cooled, then measured by weighing, and the difference in weights before and after reveals the mass of analyte lost, in this case calcium oxide. That number can then be used to calculate the amount, or the percent concentration, of it in the original mix.

Types of volatilization methods

In volatilization methods, removal of the analyte involves separation by heating or chemically decomposing a volatile sample at a suitable temperature. In other words, thermal or chemical energy is used to precipitate a volatile species. For example, the water content of a compound can be determined by vaporizing the water using thermal energy (heat). Heat can also be used, if oxygen is present, for combustion to isolate the suspect species and obtain the desired results.

The two most common gravimetric methods using volatilization are those for water and carbon dioxide. An example of this method is the isolation of sodium hydrogen bicarbonate (the main ingredient in most antacid tablets) from a mixture of

carbonate and bicarbonate. The total amount of this analyte, in whatever form, is obtained by addition of an excess of dilute sulfuric acid to the analyte in solution.

In this reaction, nitrogen gas is introduced through a tube into the flask which contains the solution. As it passes through, it gently bubbles. The gas then exits, first passing a drying agent (here $CaSO_4$, the common desiccant *Drierite*). It then passes a mixture of the drying agent *and* sodium hydroxide which lays on asbestos or *Ascarite II*, a non-fibrous silicate containing sodium hydroxide The mass of the carbon dioxide is obtained by measuring the increase in mass of this absorbent. This is performed by measuring the difference in weight of the tube in which the ascarite contained before and after the procedure.

The calcium sulfate ($CaSO_4$) in the tube retains carbon dioxide selectively as it's heated, and thereby, removed from the solution. The drying agent absorbs any aerosolized water and/or water vapor (reaction 3.). The mix of the drying agent and NaOH absorbs the CO_2 and any water that may have been produced as a result of the absorption of the NaOH (reaction 4.).

The reactions are:

Reaction 3 - absorption of water

$NaHCO_3(aq) + H_2SO_4(aq) \rightarrow CO_2(g) + H_2O(l) + NaHSO_4(aq)$.

Reaction 4. Absorption of CO_2 and residual water

$CO_2(g) + 2\ NaOH(s) \rightarrow Na_2CO_3(s) + H_2O(l)$.

Volatilization methods

Volatilization methods can be either *direct* or *indirect*. Water eliminated in a quantitative manner from many inorganic substances by ignition is an example of a direct determination. It is collected on a solid desiccant and its mass determined by the gain in mass of the desiccant.

Another direct volatilization method involves carbonates which generally decompose to release carbon dioxide when acids are used. Because carbon dioxide is easily evolved when heat is applied, its mass is directly established by the measured increase in the mass of the absorbent solid used.

Determination of the amount of water by measuring the loss in mass of the sample during heating is an example of an indirect method. It is well known that changes in mass occur due to decomposition of many substances when heat is applied, regardless of the presence or absence of water. Because one must make the assumption that water was the only component lost, this method is less satisfactory than direct methods.

This often fault and misleading assumption has proven to be wrong on more than a few occasions. There are many substances other than water loss that can lead to loss of mass with the addition of heat, as well as a number of other factors that may contribute to it. The widened margin of error created by this all-too-often false assumption is not one to be lightly disregarded as the consequences could be far-reaching.

Nevertheless, the indirect method, although less reliable than direct, is still widely used in commerce. For example, it's used

to measure the moisture content of cereals, where a number of imprecise and inaccurate instruments are available for this purpose.

Procedure

- The sample is dissolved, if it is not already in solution.
- The solution may be treated to adjust the pH (so that the proper precipitate is formed, or to suppress the formation of other precipitates). If it is known that species are present which interfere (by also forming precipitates under the same conditions as the analyte), the sample might require treatment with a different reagent to remove these interferents.
- The precipitating reagent is added at a concentration that favors the formation of a "good" precipitate (see below). This may require low concentration, extensive heating (often described as "digestion"), or careful control of the pH. Digestion can help reduce the amount of coprecipitation.
- After the precipitate has formed and been allowed to "digest", the solution is carefully filtered. The filteris used to collect the precipitate; smaller particles are more difficult to filter.
- Depending on the procedure followed, the filter might be a piece of ashlessfilter paper in a fluted funnel, or a filter crucible. Filter paper is convenient because it does not typically require cleaning before use;

however, filter paper can be chemically attacked by some solutions (such as concentrated acid or base), and may tear during the filtration of large volumes of solution.

- The alternative is a crucible whose bottom is made of some porous material, such as sintered glass, porcelain or sometimes metal. These are chemically inert and mechanically stable, even at elevated temperatures. However, they must be carefully cleaned to minimize contamination or carryover(cross-contamination). Crucibles are often used with a mat of glass or asbestos fibers to trap small particles.
- After the solution has been filtered, it should be tested to make sure that the analyte has been completely precipitated. This is easily done by adding a few drops of the precipitating reagent; if a precipitate is observed, the precipitation is incomplete.
- After filtration, the precipitate – including the filter paper or crucible – is heated, or charred. This accomplishes the following:
- The remaining moisture is removed (drying).
- Secondly, the precipitate is converted to a more chemically stable form. For instance, calcium ion might be precipitated using oxalate ion, to produce calcium oxalate (CaC_2O_4); it might then be heated to convert it into the oxide (CaO). It is vital that the empirical formula of the weighed precipitate be known, and that the precipitate be pure; if two forms are present, the results will be inaccurate.

- The precipitate cannot be weighed with the necessary accuracy in place on the filter paper; nor can the precipitate be completely removed from the filter paper to weigh it. The precipitate can be carefully heated in a crucible until the filter paper has burned away; this leaves only the precipitate. (As the name suggests, "ashless" paper is used so that the precipitate is not contaminated with ash.)
- After the precipitate is allowed to cool (preferably in a desiccator to keep it from absorbing moisture), it is weighed (in the crucible). To calculate the final mass of the analyte, the starting mass of the empty crucible is subtracted from the final mass of the crucible containing the sample. Since the composition of the precipitate is known, it is simple to calculate the mass of analyte in the original sample.

Example

A chunk of ore is to be analyzed for sulfur content. It is treated with concentrated nitric acid and potassium chlorate to convert all of the sulfur to sulfate (SO_4). The nitrate and chlorate are removed by treating the solution with concentrated HCl. The sulfate is precipitated with barium (Ba) and weighed as $BaSO_4$.

Advantages

Gravimetric analysis, if methods are followed carefully, provides for exceedingly precise analysis. In fact, gravimetric

analysis was used to determine the atomic masses of many elements in the periodic table to six figure accuracy. Gravimetry provides very little room for instrumental error and does not require a series of standards for calculation of an unknown. Also, methods often do not require expensive equipment. Gravimetric analysis, due to its high degree of accuracy, when performed correctly, can also be used to calibrate other instruments in lieu of reference standards. Gravimetric analysis is currently used to allow undergraduate chemistry/Biochemistry students to experience a grad level laboratory and it is a highly effective teaching tool to those who want to attend medical school or any research graduate school.

Disadvantages

Gravimetric analysis usually only provides for the analysis of a single element, or a limited group of elements, at a time. Comparing modern dynamic flash combustion coupled with gas chromatography with traditional combustion analysis will show that the former is both faster and allows for simultaneous determination of multiple elements while traditional determination allowed only for the determination of carbon and hydrogen. Methods are often convoluted and a slight mis-step in a procedure can often mean disaster for the analysis (colloid formation in precipitation gravimetry, for example).

Compare this with hardy methods such as spectrophotometry and one will find that analysis by these methods is much more efficient.

Steps in a gravimetric analysis

After appropriate dissolution of the sample the following steps should be followed for successful gravimetric procedure:

1. Preparation of the Solution: This may involve several steps including adjustment of the pH of the solution in order for the precipitate to occur quantitatively and get a precipitate of desired properties, removing interferences, adjusting the volume of the sample to suit the amount of precipitating agent to be added.

2. Precipitation: This requires addition of a precipitating agent solution to the sample solution. Upon addition of the first drops of the precipitating agent, supersaturation occurs, then nucleation starts to occur where every few molecules of precipitate aggregate together forming a nucleus. At this point, addition of extra precipitating agent will either form new nuclei or will build up on existing nuclei to give a precipitate. This can be predicted by Von Weimarn ratio where, according to this relation the particle size is inversely proportional to a quantity called the relative supersaturation where

Relative supersaturation = $(Q - S)/S$

The Q is the concentration of reactants before precipitation, S is the solubility of precipitate in the medium from which it is being precipitated. Therefore, to get particle growth instead of further nucleation we must make the relative supersaturation ratio as small as possible. The optimum conditions for precipitation which make the supersaturation low are:

a. Precipitation using dilute solutions to decrease Q b. Slow addition of precipitating agent to keep Q as low as possible c. Stirring the solution during addition of precipitating agent to avoid concentration sites and keep Q low d. Increase solubility by precipitation from hot solution e. Adjust the pH to increase S, but not too much increase np as we do not want to lose precipitate by dissolution f. Usually add a little excess of the precipitating agent for quantitative precipitation and check for completeness of the precipitation

3. Digestion of the precipitate: The precipitate is left hot (below boiling) for 30 min to one hour for the particles to be digested. Digestion involves dissolution of small particles and reprecipitation on larger ones resulting in particle growth and better precipitate characteristics. This process is calledOstwald ripening. An important advantage of digestion is observed for colloidal precipitates where large amounts of adsorbed ions cover the huge area of the precipitate. Digestion forces the small colloidal particles to agglomerate which decreases their surface area and thus adsorption. You should know that adsorption is a major problem in gravimetry in case of colloidal precipitate since a precipitate tends to adsorb its own ions present in excess, Therefore, forming what is called a primary ion layer which attracts ions from solution forming a secondary or counter ion layer. Individual particles repel each other keeping the colloidal properties of the precipitate. Particle coagulation can be forced by either digestion or addition of a high concentration of a diverse ions strong electrolytic solution in order to shield the charges on colloidal particles and force agglomeration. Usually, coagulated particles return to the colloidal state if washed with water, a process called peptization.

4. Washing and Filtering the Precipitate: It is crucial to wash the precipitate thoroughly to remove all adsorbed species that would add to the weight of the precipitate. One should be careful nor to use too much water since part of the precipitate may be lost. Also, in case of colloidal precipitates we should not use water as a washing solution since peptization would occur. In such situations dilute nitric acid, ammonium nitrate, or dilute acetic acid may be used. Usually, it is a good practice to check for the presence of precipitating agent in the filtrate of the final washing solution. The presence of precipitating agent means that extra washing is required. Filtration should be done in appropriate sized Gooch or ignition filter paper.

5. Drying and Ignition: The purpose of drying (heating at about 120-150 oC in an oven) or ignition in a muffle furnace at temperatures ranging from 600-1200 oC is to get a material with exactly known chemical structure so that the amount of analyte can be accurately determined.

6. Precipitation from Homogeneous Solution: To make Q minimum we can, in some situations, generate the precipitating agent in the precipitation medium rather than adding it. For example, to precipitate iron as the hydroxide, we dissolve urea in the sample. Heating of the solution generates hydroxide ions from the hydrolysis of urea. Hydroxide ions are generated at all points in solution and thus there are no sites of concentration. We can also adjust the rate of urea hydrolysis and thus control the hydroxide generation rate. This type of procedure can be very advantageous in case of colloidal precipitates.

Solubility in the presence of diverse ions

As expected from previous information, diverse ions have a screening effect on dissociated ions which leads to extra dissociation. Solubility will show a clear increase in presence of diverse ions as the solubility product will increase. Look at the following example:

Find the solubility of AgCl (K_{sp} = 1.0 x 10) in 0.1 M $NaNO_3$. The activity coefficients for silver and chloride are 0.75 and 0.76, respectively.

- AgCl(s) = Ag + Cl

We can no longer use the thermodynamic equilibrium constant (i.e. in absence of diverse ions) and we have to consider the concentration equilibrium constant or use activities instead of concentration if we use Kth:

- K_{sp} = aAgaCl
- K_{sp} = [Ag] fAg [Cl] fCl
- 1.0 x 10 = s x 0.75 x s x 0.76
- s = 1.3 x 10 M

We have calculated the solubility of AgCl in pure water to be 1.0 x 10 M, if we compare this value to that obtained in presence of diverse ions we see % increase in solubility = {(1.3 x 10 – 1.0 x 10) / 1.0 x 10} x 100 = 30% Therefore, once again we have an evidence for an increase in dissociation or a shift of equilibrium to right in presence of diverse ions.

Ion-mobility spectrometry

Ion-mobility spectrometry (**IMS**) is an analytical technique used to separate and identify ionized molecules in the gas phase based on their mobility in a carrier buffer gas. Though heavily employed for military or security purposes, such as detecting drugs and explosives, the technique also has many laboratory analytical applications, including the analysis of both small and large biomolecules. IMS instruments are extremely sensitive stand-alone devices, but are often coupled with mass spectrometry, gas chromatography or high-performance liquid chromatography in order to achieve a multi-dimensional separation. They come in various sizes, ranging from a few millimeters to several meters depending on the specific application, and are capable of operating under a broad range of conditions. IMS instruments such as microscale high-field asymmetric-waveform ion-mobility spectrometry can be palm-portable for use in a range of applications including volatile organic compound (VOC) monitoring, biological sample analysis, medical diagnosis and food quality monitoring. Systems operated at higher pressure (i.e. atmospheric conditions, 1 atm or 1013 hPa) are often accompanied by elevated temperature (above 100 °C), while lower pressure systems (1-20 hPa) do not require heating.

History

IMS was first developed primarily by Earl W. McDaniel of Georgia Institute of Technology in the 1950s and 1960s when he used drift cells with low applied electric fields to study gas phase ion mobilities and reactions. In the following decades, he

coupled his new technique with a magnetic-sector mass spectrometer, with others also utilizing his techniques in new ways. IMS cells have since been attached to many other mass spectrometers, gas chromatographs and high-performance liquid chromatography setups. IMS is a widely used technique, and improvements and other uses are continually being developed.

Applications

Perhaps ion mobility spectrometry's greatest strength is the speed at which separations occur—typically on the order of tens of milliseconds. This feature combined with its ease of use, relatively high sensitivity, and highly compact design have allowed IMS as a commercial product to be used as a routine tool for the field detection of explosives, drugs, and chemical weapons. Major manufacturers of IMS screening devices used in airports are Morpho and Smiths Detection. Smiths purchased Morpho Detection in 2017 and subsequently had to legally divest ownership of the Trace side of the business[Smiths have Trace Products] which was sold on to Rapiscan Systems in mid 2017. The products are listed under ETD Itemisers. The latest model is a non-radiation 4DX.

In the pharmaceutical industry IMS is used in cleaning validations, demonstrating that reaction vessels are sufficiently clean to proceed with the next batch of pharmaceutical product. IMS is much faster and more accurate than HPLC and total organic carbon methods previously used. IMS is also used for analyzing the composition of drugs produced, thereby finding a place in quality assurance and control.

As a research tool ion mobility is becoming more widely used in the analysis of biological materials, specifically, proteomics and metabolomics. For example, IMS-MS using MALDI as the ionization method has helped make advances in proteomics, providing faster high-resolution separations of protein pieces in analysis. Moreover, it is a really promising tool for glycomics, as rotationally averaged collision cross section (CCS) values can be obtained. CCS values are important distinguishing characteristics of ions in the gas phase, and in addition to the empirical determinations it can also be calculated computationally when the 3D structure of the molecule is known. This way, adding CCS values of glycans and their fragments to databases will increase structural identification confidence and accuracy.

Outside of laboratory purposes, IMS has found great usage as a detection tool for hazardous substances. More than 10,000 IMS devices are in use worldwide in airports, and the US Army has more than 50,000 IMS devices.

In industrial settings, uses of IMS include checking equipment cleanliness and detecting emission contents, such as determining the amount of hydrochloric and hydrofluoric acid in a stack gas from a process. It is also applied in industrial purposes to detect harmful substances in air.

In metabolomics the IMS is used to detect lung cancer, Chronic obstructive pulmonary disease, sarcoidosis, potential rejections after lung transplantation and relations to bacteria within the lung (see breath gas analysis).

Ionization

The molecules of the sample need to be ionized, usually by corona discharge, atmospheric pressure photoionization (APPI), electrospray ionization (ESI), or radioactiveatmospheric-pressure chemical ionization (R-APCI) source, e.g. a small piece of Ni or Am, similar to the one used in ionization smoke detectors. ESI and MALDI techniques are commonly used when IMS is paired with mass spectrometry.

Doping materials are sometimes added to the drift gas for ionization selectivity. For example, acetone can be added for chemical warfare agent detection, chlorinated solvents added for explosives, and nicotinamide added for drugs detection.

Analyzers

Ion mobility spectrometers exist based on various principles, optimized for different applications. A review from 2014 lists eight different ion mobility spectrometry concepts.

Drift tube

- In its simplest form, an IMS system measures how long a given ion takes to traverse a given length in a uniform electric field through a given atmosphere. In specified intervals, a sample of the ions is let into the drift chamber; the gating mechanism is based on a charged electrode working in a similar way as the control grid in triodes works for electrons. For precise control of the ion pulse width admitted to the

drift tube, more complex gating systems such as a Bradbury-Nielsen or a Field Switching Shutter are employed. Once in the drift tube, ions are subjected to a homogeneous electric field ranging from a few volts per centimeter up to many hundreds of volts per centimeter. This electric field then drives the ions through the drift tube where they interact with the neutral drift molecules contained within the system and separate based on the ion mobility, arriving at the detector for measurement. Ions are recorded at the detector in order from the fastest to the slowest, generating a response signal characteristic for the chemical composition of the measured sample.

Low pressure drift tube

Reduced pressure drift tubes operate using the same principles as their atmospheric pressure counterparts, but at drift gas pressure of only a few torr.

Due to the vastly reduced number of ion-neutral interactions, much longer drift tubes or much faster ion shutters are necessary to achieve the same resolving power. However, the reduced pressure operation offers several advantages.

First, it eases interfacing the IMS with mass spectrometry. Second, at lower pressures, ions can be stored for injection from an ion trap and re-focussed radially during and after the separation. Third, high values of E/N can be achieved, allowing for direct measurement of $K(E/N)$ over a wide range.

Travelling wave

Though drift electric fields are normally uniform, non-uniform drift fields can also be used. One example is the travelling wave IMS, which is a low pressure drift tube IMS where the electric field is only applied in a small region of the drift tube. This region then moves along the drift tube, creating a wave pushing the ions towards the detector, removing the need for a high total drift voltage. A direct determination of collision cross sections (CCS) is not possible, using TWIMS. Calibrants can help circumvent this major drawback, however, these should be matched for size, charge and chemical class of the given analyte. An especially noteworthy variant is the "SUPER" IMS, which combines ion trapping by the so-called structures for lossless ion manipulations (SLIM) with several passes through the same drift region to achieve extremely high resolving powers.

Trapped ion mobility spectrometry

In trapped ion mobility spectrometry (TIMS), ions are held stationary (or trapped) in a flowing buffer gas by an axial electric field gradient (EFG) profile while the application of radio frequency (rf) potentials results in trapping in the radial dimension. TIMS operates in the pressure range of 2 to 5 hPa and replaces the ion funnel found in the source region of modern mass spectrometers. It can be coupled with nearly any mass analyzer through either the standard mode of operation for beam-type instruments or selective accumulation mode (SA-TIMS) when used with trapping mass spectrometry (MS) instruments.

Effectively, the drift cell is prolonged by the ion motion created through the gas flow. Thus, TIMS devices do neither require large size nor high voltage in order to achieve high resolution, for instance achieving over 250 resolving power from a 4.7 cm device through the use of extended separation times. However, the resolving power strongly depends on the ion mobility and decreases for more mobile ions. In addition, TIMS can be capable of higher sensitivity than other ion mobility systems because no grids or shutters exist in the ion path, improving ion transmission both during ion mobility experiments and while operating in a transparent MS only mode.

High-field asymmetric waveform ion mobility spectrometry

DMS (differential mobility spectrometer) or FAIMS (field asymmetric ion mobility spectrometer) make use of the dependence of the ion mobility K on the electric field strength E at high electric fields. Ions are transported through the device by the drift gas flow and subjected to different field strengths in orthogonal direction for different amounts of time. Ions are deflected towards the walls of the analyzer based on the change of their mobility. Thereby only ions with a certain mobility dependence can pass the thus created filter

Differential mobility analyzer

- A **differential mobility analyzer** (**DMA**) makes use of a fast gas stream perpendicular to the electric field. Thereby ions of different mobilities undergo different trajectories. This type of IMS corresponds to the sector instruments in mass spectrometry.

They also work as a scannable filter. Examples include the differential mobility detector first commercialized by Varian in the CP-4900 MicroGC. Aspiration IMS operates with open-loop circulation of sampled air. Sample flow is passed via ionization chamber and then enters to measurement area where the ions are deflected into one or more measuring electrodes by perpendicular electric field which can be either static or varying. The output of the sensor is characteristic of the ion mobility distribution and can be used for detection and identification purposes.

A DMA can separate charged aerosol particles or ions according to their mobility in an electric field prior to their detection, which can be done with several means, including electrometers or the more sophisticated mass spectrometers.

Drift gas

The drift gas composition is an important parameter for the IMS instrument design and resolution. Often, different drift gas compositions can allow for the separation of otherwise overlapping peaks. Elevated gas temperature assists in removing ion clusters that may distort experimental measurements.

Detector

Often the detector is a simple Faraday plate coupled to a transimpedanceamplifier, however, more advanced ion mobility

instruments are coupled with mass spectrometers in order to obtain both size and mass information simultaneously. It is noteworthy that the detector influences the optimum operating conditions for the ion mobility experiment.

Combined methods

IMS can be combined with other separation techniques.

Gas chromatography

When IMS is coupled with gas chromatography, common sample introduction is with the GC capillary column directly connected to the IMS setup, with molecules ionized as they elute from GC. A similar technique is commonly used for HPLC. A novel design for corona discharge ionization ion mobility spectrometry (CD–IMS) as a detector after capillary gas chromatography has been produced in 2012. In this design, a hollow needle was used for corona discharge creation and the effluent was entered into the ionization region on the upstream side of the corona source. In addition to the practical conveniences in coupling the capillary to IMS cell, this direct axial interfacing helps us to achieve a more efficient ionization, resulting in higher sensitivity.

When used with GC, a differential mobility analyzer is often called a **differential mobility detector** (**DMD**). A DMD is often a type of microelectromechanical system, radio frequency modulated ion mobility spectrometry (MEMS RF-IMS) device. Though small, it can fit into portable units, such as transferable gas chromatographs or drug/explosives sensors. For instance, it was incorporated by Varian in its CP-4900

DMD MicroGC, and by Thermo Fisher in its EGIS Defender system, designed to detect narcotics and explosives in transportation or other security applications.

Liquid chromatography

Coupled with LC and MS, IMS has become widely used to analyze biomolecules, a practice heavily developed by David E. Clemmer, now at Indiana University (Bloomington).

Mass spectrometry

When IMS is used with mass spectrometry, ion mobility spectrometry-mass spectrometry offers many advantages, including better signal to noise, isomer separation, and charge state identification. IMS has commonly been attached to several mass spec analyzers, including quadropole, time-of-flight, and Fourier transform cyclotron resonance.

Dedicated software

Ion mobility mass spectrometry is a rather recently popularized gas phase ion analysis technique. As such there is not a large software offering to display and analyze ion mobility mass spectrometric data, apart from the software packages that are shipped along with the instruments. ProteoWizard, OpenMS, and msXpertSuite are free software according to the OpenSourceInitiative definition. While ProteoWizard and OpenMS have features to allow spectrum scrutiny, those software packages do not provide combination features. In contrast, msXpertSuite features the ability to combine spectra according to various criteria: retention time, m/z range, drift

time range, for example. msXpertSuite thus more closely mimicks the software that usually comes bundled with the mass spectrometer.

Mass spectrometry

Mass spectrometry (**MS**) is an analytical technique that is used to measure the mass-to-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.

A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds.

In a typical MS procedure, a sample, which may be solid, liquid, or gaseous, is ionized, for example by bombarding it with a beam of electrons. This may cause some of the sample's molecules to break up into positively charged fragments or simply become positively charged without fragmenting. These ions (fragments) are then separated according to their mass-to-charge ratio, for example by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection. The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the signal intensity of detected ions as a function

of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses (e.g. an entire molecule) to the identified masses or through a characteristic fragmentation pattern.

History of the mass spectrometer

- In 1886, Eugen Goldstein observed rays in gas discharges under low pressure that traveled away from the anode and through channels in a perforated cathode, opposite to the direction of negatively charged cathode rays (which travel from cathode to anode). Goldstein called these positively charged anode rays "Kanalstrahlen"; the standard translation of this term into English is "canal rays". Wilhelm Wien found that strong electric or magnetic fields deflected the canal rays and, in 1899, constructed a device with perpendicular electric and magnetic fields that separated the positive rays according to their charge-to-mass ratio (Q/m). Wien found that the charge-to-mass ratio depended on the nature of the gas in the discharge tube. English scientist J. J. Thomson later improved on the work of Wien by reducing the pressure to create the mass spectrograph.

The word *spectrograph* had become part of the international scientific vocabulary by 1884. Early *spectrometry* devices that measured the mass-to-charge ratio of ions were called *mass spectrographs* which consisted of instruments that recorded a spectrum of mass values on a photographic plate. A *mass spectroscope* is similar to a *mass spectrograph* except that the

beam of ions is directed onto a phosphor screen. A mass spectroscope configuration was used in early instruments when it was desired that the effects of adjustments be quickly observed. Once the instrument was properly adjusted, a photographic plate was inserted and exposed. The term mass spectroscope continued to be used even though the direct illumination of a phosphor screen was replaced by indirect measurements with an oscilloscope. The use of the term *mass spectroscopy* is now discouraged due to the possibility of confusion with light spectroscopy. Mass spectrometry is often abbreviated as *mass-spec* or simply as *MS*.

Modern techniques of mass spectrometry were devised by Arthur Jeffrey Dempster and F.W. Aston in 1918 and 1919 respectively.

Sector mass spectrometers known as calutronswere developed by Ernest O. Lawrence and used for separating the isotopes of uranium during the Manhattan Project. Calutron mass spectrometers were used for uranium enrichment at the Oak Ridge, TennesseeY-12 plant established during World War II.

In 1989, half of the Nobel Prize in Physicswas awarded to Hans Dehmelt and Wolfgang Paul for the development of the ion trap technique in the 1950s and 1960s.

In 2002, the Nobel Prize in Chemistrywas awarded to John Bennett Fenn for the development of electrospray ionization (ESI) and Koichi Tanaka for the development of soft laser desorption (SLD) and their application to the ionization of biological macromolecules, especially proteins.

Parts of a mass spectrometer

A mass spectrometer consists of three components: an ion source, a mass analyzer, and a detector. The *ionizer* converts a portion of the sample into ions. There is a wide variety of ionization techniques, depending on the phase (solid, liquid, gas) of the sample and the efficiency of various ionization mechanisms for the unknown species. An extraction system removes ions from the sample, which are then targeted through the mass analyzer and into the *detector*. The differences in masses of the fragments allows the mass analyzer to sort the ions by their mass-to-charge ratio. The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. Some detectors also give spatial information, e.g., a multichannel plate.

Theoretical 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 u. Chloride atoms and ions come in two isotopes with masses of approximately 35 u (at a natural abundance of about 75 percent) and approximately 37 u (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 Cl to Cl).

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 ionizationare used for gases and vapors. In chemical ionization sources, the analyteis 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).

Hard ionization and soft ionization

In mass spectrometry, ionization refers to the production of gas phase ions suitable for resolution in the mass analyser or mass filter. Ionization occurs in the ion source. There are several ion sources available; each has advantages and disadvantages for particular applications. For example, electron ionization (EI) gives a high degree of fragmentation, yielding highly detailed mass spectra which when skilfullyanalysed can provide important information for structural elucidation/characterisation and facilitate identification of unknown compounds by comparison to mass spectral libraries obtained under identical operating conditions. However, EI is not suitable for coupling to HPLC, i.e. LC-MS, since at atmospheric pressure, the filaments used to generate electrons burn out rapidly. Thus EI is coupled predominantly with GC, i.e. GC-MS, where the entire system is under high vacuum.

Hard ionization techniques are processes which impart high quantities of residual energy in the subject molecule invoking large degrees of fragmentation (i.e. the systematic rupturing of bonds acts to remove the excess energy, restoring stability to the resulting ion). Resultant ions tend to have m/z lower than the molecular mass (other than in the case of proton transfer and not including isotope peaks). The most common example of hard ionization is electron ionization (EI).

Soft ionization refers to the processes which impart little residual energy onto the subject molecule and as such result in little fragmentation. Examples include fast atom bombardment (FAB), chemical ionization (CI), atmospheric-

pressure chemical ionization (APCI), atmospheric-pressure photoionization (APPI), electrospray ionization (ESI), desorption electrospray ionization (DESI), and matrix-assisted laser desorption/ionization (MALDI).

Inductively coupled plasma

Inductively coupled plasma (ICP) sources are used primarily for cation analysis of a wide array of sample types. In this source, a 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, 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.

Photoionization mass spectrometry

Photoionization can be used in experiments which seek to use mass spectrometry as a means of resolving chemical kinetics mechanisms and isomeric product branching. In such instances a high energy photon, either X-ray or uv, is used to dissociate stable gaseous molecules in a carrier gas of He or Ar. In instances where a synchrotron light source is utilized, a tuneable photon energy can be utilized to acquire a photoionization efficiency curve which can be used in conjunction with the charge ratio m/z to fingerprint molecular and ionic species. More recentlyatmospheric pressure

photoionization (APPI) has been developed to ionize molecules mostly as effluents of LC-MS systems.

Ambient ionization

Some applications for ambient ionization include environmental applications as well as clinical applications. In these techniques, ions form in an ion source outside the mass spectrometer. Sampling becomes easy as the samples don't need previous separation nor preparation. Some examples of ambient ionization techniques are DESI, SESI, LAESI, desorption atmospheric-pressure chemical ionization (DAPCI), and desorption atmospheric pressure photoionization DAPPI among others.

Other ionization techniques

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).

Sector instruments

A sector field mass analyzer uses a static electric and/or magnetic field to affect the path and/or velocity of the charged particles in some way. As shown above, sector instruments bend the trajectories of the ions as they pass through the mass analyzer, according to their mass-to-charge ratios, deflecting the more charged and faster-moving, lighter ions more. The

analyzer can be used to select a narrow range of m/z or to scan through a range of m/z to catalog the ions present.

Time-of-flight

The time-of-flight (TOF) analyzer uses an electric field to accelerate the ions through the same potential, and then measures the time they take to reach the detector. If the particles all have the same charge, their kinetic energies will be identical, and their velocities will depend only on their masses. Ions with a lower mass will reach the detector first. However, in reality, even particles with the same m/z can arrive at different times at the detector, because they have different initial velocities. The initial velocity is often not dependent on the mass of the ion TOF-MS, and will turn into a difference in the final velocity. Because of this, ions with the same m/z ratio will reach the detector at a variety of times, which broadens the peaks shown on the count vs m/z plot, but will generally not change the central location of the peaks, since the average starting velocity of ions relative to the other analyzed ions is generally centered at zero. To fix this problem, time-lag focusing/delayed extractionhas been coupled with TOF-MS.

Quadrupole mass filter

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 magnetically enhanced quadrupole mass analyzer includes the addition of a magnetic field, either applied axially or transversely. This novel type of instrument leads to an additional performance enhancement in terms of resolution and/or sensitivity depending upon the magnitude and orientation of the applied magnetic field. 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.

Ion traps

Three-dimensional quadrupole ion trap

The quadrupole ion trap works on the same physical principles as the quadrupole mass analyzer, but the ions are trapped and sequentially ejected. Ions are trapped in a mainly quadrupole

RF field, in a space defined by a ring electrode (usually connected to the main RF potential) between two endcap electrodes (typically connected to DC or auxiliary AC potentials). The sample is ionized either internally (e.g. with an electron or laser beam), or externally, in which case the ions are often introduced through an aperture in an endcap electrode.

There are many mass/charge separation and isolation methods but the most commonly used is the mass instability mode in which the RF potential is ramped so that the orbit of ions with a mass $a>b$ are stable while ions with mass b become unstable and are ejected on the z-axis onto a detector. There are also non-destructive analysis methods.

Ions may also be ejected by the resonance excitation method, whereby a supplemental oscillatory excitation voltage is applied to the endcap electrodes, and the trapping voltage amplitude and/or excitation voltage frequency is varied to bring ions into a resonance condition in order of their mass/charge ratio.

Cylindrical ion trap

The cylindrical ion trap mass spectrometer (CIT) is a derivative of the quadrupole ion trap where the electrodes are formed from flat rings rather than hyperbolic shaped electrodes. The architecture lends itself well to miniaturization because as the size of a trap is reduced, the shape of the electric field near the center of the trap, the region where the ions are trapped, forms a shape similar to that of a hyperbolic trap.

Linear quadrupole ion trap

A linear quadrupole ion trap is similar to a quadrupole ion trap, but it traps ions in a two dimensional quadrupole field, instead of a three-dimensional quadrupole field as in a 3D quadrupole ion trap. Thermo Fisher's LTQ ("linear trap quadrupole") is an example of the linear ion trap.

A toroidal ion trap can be visualized as a linear quadrupole curved around and connected at the ends or as a cross-section of a 3D ion trap rotated on edge to form the toroid, donut-shaped trap. The trap can store large volumes of ions by distributing them throughout the ring-like trap structure. This toroidal shaped trap is a configuration that allows the increased miniaturization of an ion trap mass analyzer. Additionally, all ions are stored in the same trapping field and ejected together simplifying detection that can be complicated with array configurations due to variations in detector alignment and machining of the arrays.

As with the toroidal trap, linear traps and 3D quadrupole ion traps are the most commonly miniaturized mass analyzers due to their high sensitivity, tolerance for mTorr pressure, and capabilities for single analyzer tandem mass spectrometry (e.g. product ion scans).

Orbitrap

Orbitrap instruments are similar to Fourier transform ion cyclotron resonance mass spectrometers (see text below). Ions are electrostatically trapped in an orbit around a central, spindle shaped electrode. The electrode confines the ions so

that they both orbit around the central electrode and oscillate back and forth along the central electrode's long axis. This oscillation generates an image current in the detector plates which is recorded by the instrument. The frequencies of these image currents depend on the mass-to-charge ratios of the ions. Mass spectra are obtained by Fourier transformation of the recorded image currents.

Orbitraps have a high mass accuracy, high sensitivity and a good dynamic range.

Fourier transform ion cyclotron resonance

Fourier transform mass spectrometry (FTMS), or more precisely Fourier transform ion cyclotron resonance MS, measures mass by detecting the image current produced by ions cyclotroning in the presence of a magnetic field. Instead of measuring the deflection of ions with a detector such as an electron multiplier, the ions are injected into a Penning trap (a static electric/magnetic ion trap) where they effectively form part of a circuit. Detectors at fixed positions in space measure the electrical signal of ions which pass near them over time, producing a periodic signal. Since the frequency of an ion's cycling is determined by its mass-to-charge ratio, this can be deconvoluted by performing a Fourier transform on the signal. FTMS has the advantage of high sensitivity (since each ion is "counted" more than once) and much higher resolution and thus precision.

Ion cyclotron resonance (ICR) is an older mass analysis technique similar to FTMS except that ions are detected with a traditional detector. Ions trapped in a Penning trap are excited

by an RF electric field until they impact the wall of the trap, where the detector is located. Ions of different mass are resolved according to impact time.

Detectors

The final element of the mass spectrometer is the detector. The detector records either the charge induced or the current produced when an ion passes by or hits a surface. In a scanning instrument, the signal produced in the detector during the course of the scan versus where the instrument is in the scan (at what m/Q) will produce a mass spectrum, a record of ions as a function of m/Q.

Typically, some type of electron multiplieris used, though other detectors including Faraday cups and ion-to-photon detectors are also used. Because the number of ions leaving the mass analyzer at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. Microchannel plate detectorsare commonly used in modern commercial instruments. In FTMS and Orbitraps, the detector consists of a pair of metal surfaces within the mass analyzer/ion trap region which the ions only pass near as they oscillate. No direct current is produced, only a weak AC image current is produced in a circuit between the electrodes. Other inductive detectors have also been used.

Tandem mass spectrometry

A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry, usually separated by some form of

molecule fragmentation. For example, one mass analyzer can isolate one peptide from many entering a mass spectrometer. A second mass analyzer then stabilizes the peptide ions while they collide with a gas, causing them to fragment by collision-induced dissociation (CID). A third mass analyzer then sorts the fragments produced from the peptides. Tandem MS can also be done in a single mass analyzer over time, as in a quadrupole ion trap. There are various methods for fragmenting molecules for tandem MS, including collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD), blackbody infrared radiative dissociation (BIRD), electron-detachment dissociation (EDD) and surface-induced dissociation (SID). An important application using tandem mass spectrometry is in protein identification.

Tandem mass spectrometry enables a variety of experimental sequences. Many commercial mass spectrometers are designed to expedite the execution of such routine sequences as selected reaction monitoring (SRM) and precursor ion scanning. In SRM, the first analyzer allows only a single mass through and the second analyzer monitors for multiple user-defined fragment ions. SRM is most often used with scanning instruments where the second mass analysis event is duty cycle limited. These experiments are used to increase specificity of detection of known molecules, notably in pharmacokinetic studies. Precursor ion scanning refers to monitoring for a specific loss from the precursor ion. The first and second mass analyzers scan across the spectrum as partitioned by a user-defined m/z value. This experiment is used to detect specific motifs within unknown molecules.

Another type of tandem mass spectrometry used for radiocarbon dating is accelerator mass spectrometry (AMS), which uses very high voltages, usually in the mega-volt range, to accelerate negative ions into a type of tandem mass spectrometer.

The **METLIN Metabolite and Chemical Entity Database** is the largest repository of experimental tandem mass spectrometry data acquired from standards. The tandem mass spectrometry data on over 850,000 molecular standards (as of 24 August 2020) is provided to facilitate the identification of chemical entities from tandem mass spectrometry experiments. In addition to the identification of known molecules it is also useful for identifying unknowns using its similarity searching/analysis. All tandem mass spectrometry data comes from the experimental analysis of standards at multiple collision energies and in both positive and negative ionization modes.

Common mass spectrometer configurations and techniques

When a specific combination of source, analyzer, and detector becomes conventional in practice, a compound acronym may arise to designate it succinctly. One example is MALDI-TOF, which refers to a combination of a matrix-assisted laser desorption/ionization source with a time-of-flight mass analyzer. Other examples include inductively coupled plasma-mass spectrometry (ICP-MS), accelerator mass spectrometry (AMS), thermal ionization-mass spectrometry (TIMS) and spark source mass spectrometry (SSMS).

Certain applications of mass spectrometry have developed monikers that although strictly speaking would seem to refer to a broad application, in practice have come instead to connote a specific or a limited number of instrument configurations. An example of this is isotope ratio mass spectrometry (IRMS), which refers in practice to the use of a limited number of sector based mass analyzers; this name is used to refer to both the application and the instrument used for the application.

Separation techniques combined with mass spectrometry

An important enhancement to the mass resolving and mass determining capabilities of mass spectrometry is using it in tandem with chromatographic and other separation techniques.

Gas chromatography

A common combination is gas chromatography-mass spectrometry (GC/MS or GC-MS). In this technique, a gas chromatographis used to separate different compounds. This stream of separated compounds is fed online into the ion source, a metallicfilament to which voltage is applied. This filament emits electrons which ionize the compounds. The ions can then further fragment, yielding predictable patterns. Intact ions and fragments pass into the mass spectrometer's analyzer and are eventually detected. However, the high temperatures (300°C) used in the GC-MS injection port (and oven) can result in thermal degradation of injected molecules, thus resulting in

the measurement of degradation products instead of the actual molecule(s) of interest.

Liquid chromatography

Similar to gas chromatography MS (GC-MS), liquid chromatography-mass spectrometry (LC/MS or LC-MS) separates compounds chromatographically before they are introduced to the ion source and mass spectrometer. It differs from GC-MS in that the mobile phase is liquid, usually a mixture of water and organic solvents, instead of gas. Most commonly, an electrospray ionization source is used in LC-MS. Other popular and commercially available LC-MS ion sources are atmospheric pressure chemical ionization and atmospheric pressure photoionization. There are also some newly developed ionization techniques like laser spray.

Capillary electrophoresis–mass spectrometry

Capillary electrophoresis–mass spectrometry (CE-MS) is a technique that combines the liquid separation process of capillary electrophoresis with mass spectrometry. CE-MS is typically coupled to electrospray ionization.

Ion mobility

Ion mobility spectrometry-mass spectrometry (IMS/MS or IMMS) is a technique where ions are first separated by drift time through some neutral gas under an applied electrical potential gradient before being introduced into a mass spectrometer. Drift time is a measure of the radius relative to the charge of the ion. The duty cycle of IMS (the time over

which the experiment takes place) is longer than most mass spectrometric techniques, such that the mass spectrometer can sample along the course of the IMS separation. This produces data about the IMS separation and the mass-to-charge ratio of the ions in a manner similar to LC-MS.

The duty cycle of IMS is short relative to liquid chromatography or gas chromatography separations and can thus be coupled to such techniques, producing triple modalities such as LC/IMS/MS.

Data and analysis

Data representations

Mass spectrometry produces various types of data. The most common data representation is the mass spectrum.

Certain types of mass spectrometry data are best represented as a mass chromatogram. Types of chromatograms include selected ion monitoring (SIM), total ion current (TIC), and selected reaction monitoring (SRM), among many others.

Other types of mass spectrometry data are well represented as a three-dimensional contour map. In this form, the mass-to-charge, m/z is on the x-axis, intensity the y-axis, and an additional experimental parameter, such as time, is recorded on the z-axis.

Data analysis

Mass spectrometry data analysis is specific to the type of experiment producing the data. General subdivisions of data are fundamental to understanding any data.

Many mass spectrometers work in either *negative ion mode* or *positive ion mode*. It is very important to know whether the observed ions are negatively or positively charged. This is often important in determining the neutral mass but it also indicates something about the nature of the molecules.

Different types of ion source result in different arrays of fragments produced from the original molecules. An electron ionization source produces many fragments and mostly single-charged (1-) radicals (odd number of electrons), whereas an electrospray source usually produces non-radical quasimolecular ions that are frequently multiply charged. Tandem mass spectrometry purposely produces fragment ions post-source and can drastically change the sort of data achieved by an experiment.

Knowledge of the origin of a sample can provide insight into the component molecules of the sample and their fragmentations. A sample from a synthesis/manufacturing process will probably contain impurities chemically related to the target component. A crudely prepared biological sample will probably contain a certain amount of salt, which may form adducts with the analyte molecules in certain analyses.

Results can also depend heavily on sample preparation and how it was run/introduced. An important example is the issue of which matrix is used for MALDI spotting, since much of the

energetics of the desorption/ionization event is controlled by the matrix rather than the laser power. Sometimes samples are spiked with sodium or another ion-carrying species to produce adducts rather than a protonated species.

Mass spectrometry can measure molar mass, molecular structure, and sample purity. Each of these questions requires a different experimental procedure; therefore, adequate definition of the experimental goal is a prerequisite for collecting the proper data and successfully interpreting it.

Interpretation of mass spectra

Since the precise structure or peptide sequence of a molecule is deciphered through the set of fragment masses, the interpretation of mass spectra requires combined use of various techniques. Usually the first strategy for identifying an unknown compound is to compare its experimental mass spectrum against a library of mass spectra. If no matches result from the search, then manual interpretation or software assisted interpretation of mass spectramust be performed. Computer simulation of ionization and fragmentation processes occurring in mass spectrometer is the primary tool for assigning structure or peptide sequence to a molecule. An *a priori* structural information is fragmented*in silico* and the resulting pattern is compared with observed spectrum. Such simulation is often supported by a fragmentation library that contains published patterns of known decomposition reactions. Software taking advantage of this idea has been developed for both small molecules and proteins.

Analysis of mass spectra can also be spectra with accurate mass. A mass-to-charge ratio value (m/z) with only integer precision can represent an immense number of theoretically possible ion structures; however, more precise mass figures significantly reduce the number of candidate molecular formulas. A computer algorithm called formula generator calculates all molecular formulas that theoretically fit a given mass with specified tolerance.

A recent technique for structure elucidation in mass spectrometry, called precursor ion fingerprinting, identifies individual pieces of structural information by conducting a search of the tandem spectra of the molecule under investigation against a library of the product-ion spectra of structurally characterized precursor ions.

Applications

Mass spectrometry 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 commonly used in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

As an analytical technique it possesses distinct advantages such as: Increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter,

reduces background interference, Excellent specificity from characteristic fragmentation patterns to identify unknowns or confirm the presence of suspected compounds, Information about molecular weight, Information about the isotopic abundance of elements, Temporally resolved chemical data.

A few of the disadvantages of the method is that it often fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p- positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.

Isotope ratio MS: isotope dating and tracing

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 spectrometers (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). This is because it provides ultimate sensitivity, capable of measuring individual atoms and measuring nuclides with a dynamic range of ~10 relative to the major stable isotope. 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. (seeprotein characterization below)

Membrane-introduction mass spectrometry: measuring gases in solution

Membrane-introduction mass spectrometry combines the isotope ratio MS with a reaction chamber/cell separated by a gas-permeable membrane. This method allows the study of gases as they evolve in solution. This method has been extensively used for the study of the production of oxygen by Photosystem II.

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.

Another technique with applications in trace gas analysis field is secondary electrospray ionization (SESI-MS), which is a variant of electrospray ionization. SESI consist of an electrospray plume of pure acidified solvent that interacts with neutral vapors. Vapor molecules get ionized at atmospheric pressure when charge is transferred from the ions formed in the electrospray to the molecules. One advantage of this approach is that it is compatible with most ESI-MS systems.

Atom probe

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

Pharmacokinetics

Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix (often blood or urine) and the need for high sensitivity to observe low dose and long time point data. The most common instrumentation used in this application is LC-MS with a triple quadrupole mass spectrometer. Tandem mass spectrometry is usually employed for added specificity. Standard curves and internal standards are used for quantitation of usually a single pharmaceutical in the samples. The samples represent different time points as a pharmaceutical is administered and then metabolized or cleared from the body. Blank or t=0 samples taken before administration are important in determining background and ensuring data integrity with such complex sample matrices. Much attention is paid to the linearity of the standard curve; however it is not uncommon to use curve fitting with more complex functions such as quadratics since the response of most mass spectrometers is less than linear across large concentration ranges.

There is currently considerable interest in the use of very high sensitivity mass spectrometry for microdosing studies, which are seen as a promising alternative to animal experimentation.

Recent studies show that secondary electrospray ionization (SESI) is a powerful technique to monitor drug kinetics via breath analysis. Because breath is naturally produced, several datapoints can be readily collected. This allows for the number of collected data-points to be greatly increased. In animal studies, this approach SESI can reduce animal sacrifice. In humans, SESI-MS non-invasive analysis of breath can help study the kinetics of drugs at a personalized level.

Protein characterization

Mass spectrometry is an important method for the characterization and sequencing of proteins. The two primary methods for ionization of whole proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). In keeping with the performance and mass range of available mass spectrometers, two approaches are used for characterizing proteins. In the first, intact proteins are ionized by either of the two techniques described above, and then introduced to a mass analyzer. This approach is referred to as "top-down" strategy of protein analysis. The top-down approach however is largely limited to low-throughput single-protein studies. In the second, proteins are enzymatically digested into smaller peptides using proteases such as trypsin or pepsin, either in solution or in gel after electrophoretic separation. Other proteolytic agents are also used. The collection of peptide products are often separated by chromatography prior to introduction to the mass analyzer. When the characteristic pattern of peptides is used for the identification of the protein the method is called peptide mass fingerprinting (PMF), if the identification is performed using the sequence data determined in tandem MS

analysis it is called de novo peptide sequencing. These procedures of protein analysis are also referred to as the "bottom-up" approach, and have also been used to analyse the distribution and position of post-translational modifications such as phosphorylation on proteins. A third approach is also beginning to be used, this intermediate "middle-down" approach involves analyzing proteolytic peptides that are larger than the typical tryptic peptide.

Space exploration

As a standard method for analysis, mass spectrometers have reached other planets and moons. Two were taken to Mars by the Viking program. In early 2005 the Cassini–Huygens mission delivered a specialized GC-MS instrument aboard the Huygens probe through the atmosphere of Titan, the largest moon of the planet Saturn. This instrument analyzed atmospheric samples along its descent trajectory and was able to vaporize and analyze samples of Titan's frozen, hydrocarbon covered surface once the probe had landed. These measurements compare the abundance of isotope(s) of each particle comparatively to earth's natural abundance. Also on board the Cassini–Huygens spacecraft was an ion and neutral mass spectrometer which had been taking measurements of Titan's atmospheric composition as well as the composition of Enceladus' plumes. A Thermal and Evolved Gas Analyzer mass spectrometer was carried by the Mars Phoenix Lander launched in 2007.

Mass spectrometers are also widely used in space missions to measure the composition of plasmas. For example, the Cassini

spacecraft carried the Cassini Plasma Spectrometer (CAPS), which measured the mass of ions in Saturn's magnetosphere.

Respired gas monitor

Mass spectrometers were used in hospitals for respiratory gas analysis beginning around 1975 through the end of the century. Some are probably still in use but none are currently being manufactured.

Found mostly in the operating room, they were a part of a complex system, in which respired gas samples from patients undergoing anesthesiawere drawn into the instrument through a valve mechanism designed to sequentially connect up to 32 rooms to the mass spectrometer. A computer directed all operations of the system. The data collected from the mass spectrometer was delivered to the individual rooms for the anesthesiologist to use.

The uniqueness of this magnetic sector mass spectrometer may have been the fact that a plane of detectors, each purposely positioned to collect all of the ion species expected to be in the samples, allowed the instrument to simultaneously report all of the gases respired by the patient. Although the mass range was limited to slightly over 120 u, fragmentation of some of the heavier molecules negated the need for a higher detection limit.

Preparative mass spectrometry

The primary function of mass spectrometry is as a tool for chemical analyses based on detection and quantification of ions according to their mass-to-charge ratio. However, mass

spectrometry also shows promise for material synthesis. Ion soft landing is characterized by deposition of intact species on surfaces at low kinetic energies which precludes the fragmentation of the incident species. The soft landing technique was first reported in 1977 for the reaction of low energy sulfur containing ions on a lead surface.

Spectroscopy

Spectroscopy is the study of the interaction between matter and electromagneticradiation as a function of the wavelength or frequency of the radiation. In simpler terms, spectroscopy is the precise study of color as generalized from visible light to all bands of the electromagnetic spectrum; indeed, historically, spectroscopy originated as the study of the wavelength dependence of the absorption by gas phase matter of visible light dispersed by a prism. Matter waves and acoustic wavescan also be considered forms of radiative energy, and recently gravitational waves have been associated with a spectral signature in the context of the Laser Interferometer Gravitational-Wave Observatory (LIGO).

Spectroscopy, primarily in the electromagnetic spectrum, is a fundamental exploratory tool in the fields of physics, chemistry, and astronomy, allowing the composition, physical structure and electronic structure of matter to be investigated at the atomic, molecular and macro scale, and over astronomical distances. Important applications arise from biomedical spectroscopy in the areas of tissue analysis and medical imaging.

Spectroscopy and spectrographyare 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.

Daily observations of colorcan be related to spectroscopy. Neon lighting is a direct application of atomic spectroscopy. Neon and other noble gases have characteristic emission frequencies (colors). Neon lamps use collision of electrons with the gas 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.

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. As a result, these spectra can be used to detect, identify and quantify information about the atoms and molecules. Spectroscopy is also used in astronomy and remote sensing on Earth. 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).

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 a photon. 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 ν by $E = h\nu$ 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. Named series of lines include the principal, sharp, diffuse and fundamental series.

Classification of methods

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.

Type of radiative energy

The 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, ultraviolet-visible, x-ray, and gamma spectroscopy.

- Particles, because of their de Broglie waves, can also be a source of radiative energy. Both electron and neutron spectroscopy are commonly used. For a particle, its kinetic energy determines its wavelength.
- Acoustic spectroscopy involves radiated pressure waves.
- Dynamic mechanical analysiscan be employed to impart radiating energy, similar to acoustic waves, to solid materials.

Nature of the interaction

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

- Absorption spectroscopy: 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, with absorption decreasing the transmitted portion.
- Emission spectroscopy: Emission indicates that radiative energy is released by the material. A material's blackbody spectrum is a spontaneous emission spectrum determined by its temperature. This feature can be measured in the infrared by instruments such as the atmospheric emitted radiance interferometer. Emission can also be

induced by other sources of energy such as flames, sparks, electric arcs 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: Impedance is the ability of a medium to impede or slow the transmittance of energy. For optical applications, this is characterized by the index of refraction.
- Inelastic 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 spectroscopyis also possible in the infrared and visible spectral regions.
- Nuclear spectroscopy are methods that use the properties of specific nuclei to probe the local structure in matter, mainly condensed matter,

molecules in liquids or frozen liquids and bio-molecules.

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 and atomic emission spectroscopy involve visible and ultraviolet light. These absorptions and emissions, often referred to as atomic spectral lines, are due to electronic transitions of outer shell electrons as they rise and fall from one electron orbit to another. 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. After inventing the spectroscope, Robert Bunsen 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 explained the Lamb shift observed in the hydrogen spectrum, which further 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.

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 nuclear magnetic resonance spectroscopy.

Other types

Other types of spectroscopy are distinguished by specific applications or implementations:

- Acoustic resonance spectroscopyis based on sound waves primarily in the audible and ultrasonic regions.
- Auger electron spectroscopy is a method used to study surfaces of materials on a micro-scale. It is often used in connection with electron microscopy.
- Cavity ring-down spectroscopy
- Circular Dichroism spectroscopy

- Coherent anti-Stokes Raman spectroscopy is a recent technique that has high sensitivity and powerful applications for *in vivo* spectroscopy and imaging.
- Cold vapour atomic fluorescence spectroscopy
- Correlation spectroscopy encompasses several types of two-dimensional NMR spectroscopy.
- Deep-level transient spectroscopy measures concentration and analyzes parameters of electrically active defects in semiconducting materials.
- Dielectric spectroscopy
- Dual-polarization interferometry measures the real and imaginary components of the complex refractive index.
- Electron energy loss spectroscopy in transmission electron microscopy.
- Electron phenomenological spectroscopy measures the physicochemical properties and characteristics of the electronic structure of multicomponent and complex molecular systems.
- Electron paramagnetic resonance spectroscopy
- Force spectroscopy
- Fourier-transform spectroscopy is an efficient method for processing spectra data obtained using interferometers. Fourier-transform infrared spectroscopy is a common implementation of infrared spectroscopy. NMR also employs Fourier transforms.
- Hadron spectroscopy studies the energy/mass spectrum of hadrons according to spin, parity, and other particle properties. Baryon spectroscopy and meson spectroscopy are types of hadron spectroscopy.

- Hyperspectral imaging is a method to create a complete picture of the environment or various objects, each pixel containing a full visible, visible near infrared, near infrared, or infrared spectrum.
- Inelastic electron tunneling spectroscopy uses the changes in current due to inelastic electron-vibration interaction at specific energies that can also measure optically forbidden transitions.
- Inelastic neutron scattering is similar to Raman spectroscopy, but uses neutrons instead of photons.
- Laser-induced breakdown spectroscopy, also called laser-induced plasma spectrometry
- Laser spectroscopy uses tunable lasers and other types of coherent emission sources, such as optical parametric oscillators, for selective excitation of atomic or molecular species.
- Mass spectroscopy is a historical term used to refer to mass spectrometry. The current recommendation is to use the latter term. The term "mass spectroscopy" originated in the use of phosphor screens to detect ions.
- Mössbauer spectroscopy probes the properties of specific isotopicnuclei in different atomic environments by analyzing the resonant absorption of gamma rays. See also Mössbauer effect.
- Multivariate optical computing is an all opticalcompressed sensing technique, generally used in harsh environments, that directly calculates chemical information from a spectrum as analogue output.
- Neutron spin echo spectroscopy measures internal dynamics in proteins and other soft matter systems.

- Perturbed angular correlation (PAC) uses radioactive nuclei as probe to study electric and magnetic fields (hyperfine interactions) in crystals (condensed matter) and bio-molecules.
- Photoacoustic spectroscopy measures the sound waves produced upon the absorption of radiation.
- Photoemission spectroscopy
- Photothermal spectroscopy measures heat evolved upon absorption of radiation.
- Pump-probe spectroscopy can use ultrafast laser pulses to measure reaction intermediates in the femtosecond timescale.
- Raman optical activity spectroscopy exploits Raman scattering and optical activity effects to reveal detailed information on chiral centers in molecules.
- Raman spectroscopy
- Saturated spectroscopy
- Scanning tunneling spectroscopy
- Spectrophotometry
- Spin noise spectroscopy traces spontaneous fluctuations of electronic and nuclear spins.
- Time-resolved spectroscopy measures the decay rates of excited states using various spectroscopic methods.
- Time-stretch spectroscopy
- Thermal infrared spectroscopy measures thermal radiation emitted from materials and surfaces and is used to determine the type of bonds present in a sample as well as their lattice environment. The techniques are widely used by organic chemists, mineralogists, and planetary scientists.

- Transient grating spectroscopy measures quasiparticle propagation. It can track changes in metallic materials as they are irradiated.
- Ultraviolet photoelectron spectroscopy
- Ultraviolet–visible spectroscopy
- Vibrational circular dichroism spectroscopy
- Video spectroscopy
- X-ray photoelectron spectroscopy

Applications

There are several applications to spectroscopy in the field of medicine, physics, chemistry, and astronomy. Taking advantage of the properties of absorbance, spectroscopy can be used to identify certain states of nature. Such examples include:

- Cure monitoring of composites using optical fibers.
- Estimate weathered wood exposure times using near infrared spectroscopy.
- Measurement of different compounds in food samples by absorption spectroscopy both in visible and infrared spectrum.
- Measurement of toxic compounds in blood samples
- Non-destructive elemental analysis by X-ray fluorescence.
- Electronic structure research with various spectroscopes.
- Radar to determine the speed and velocity of a distant object

- Finding the physical properties of a distant star or nearby exoplanet using the Relativistic Doppler effect.

History

The history of spectroscopy began with Isaac Newton's optics experiments (1666–1672). According to Andrew Fraknoi and David Morrison, "In 1672, in the first paper that he submitted to the Royal Society, Isaac Newton described an experiment in which he permitted sunlight to pass through a small hole and then through a prism. Newton found that sunlight, which looks white to us, is actually made up of a mixture of all the colors of the rainbow." Newton applied the word "spectrum" to describe the rainbow of colors that combine to form white light and that are revealed when the white light is passed through a prism.

Fraknoi and Morrison state that "In 1802, William Hyde Wollaston built an improved spectrometer that included a lens to focus the Sun's spectrum on a screen. Upon use, Wollaston realized that the colors were not spread uniformly, but instead had missing patches of colors, which appeared as dark bands in the spectrum." During the early 1800s, Joseph von Fraunhofer made experimental advances with dispersive spectrometers that enabled spectroscopy to become a more precise and quantitative scientific technique. Since then, spectroscopy has played and continues to play a significant role in chemistry, physics, and astronomy. Per Fraknoi and Morrison, "Later, in 1815, German physicist Joseph Fraunhofer also examined the solar spectrum, and found about 600 such

dark lines (missing colors), are now known as Fraunhofer lines, or Absorption lines."

Titration

Titration (also known as **titrimetry** and **volumetric analysis**) is a common laboratory method of quantitativechemical analysis to determine the concentration of an identified analyte (a substance to be analyzed). A reagent, termed the *titrant* or *titrator*, is prepared as a standard solution of known concentration and volume. The titrant reacts with a solution of *analyte* (which may also be termed the *titrand*) to determine the analyte's concentration. The volume of titrant that reacted with the analyte is termed the *titration volume*.

History and etymology

The word "titration" descends from the French word *tiltre* (1543), meaning the proportion of gold or silver in coins or in works of gold or silver; i.e., a measure of fineness or purity. *Tiltre* became *titre*, which thus came to mean the "fineness of alloyed gold", and then the "concentration of a substance in a given sample". In 1828, the French chemist Joseph Louis Gay-Lussac first used *titre* as a verb (*titrer*), meaning "to determine the concentration of a substance in a given sample".

Volumetric analysis originated in late 18th-century France. François-Antoine-Henri Descroizilles (fr) developed the first burette (which was similar to a graduated cylinder) in 1791. Gay-Lussac developed an improved version of the burette that included a side arm, and invented the terms "pipette" and

"burette" in an 1824 paper on the standardization of indigo solutions. The first true burette was invented in 1845 by the French chemist Étienne Ossian Henry (1798–1873). A major improvement of the method and popularization of volumetric analysis was due to Karl Friedrich Mohr, who redesigned the burette into a simple and convenient form, and who wrote the first textbook on the topic, *Lehrbuch der chemisch-analytischenTitrirmethode* (*Textbook of analytical chemistry titration methods*), published in 1855.

Procedure

A typical titration begins with a beaker or Erlenmeyer flask containing a very precise amount of the analyte and a small amount of indicator (such as phenolphthalein) placed underneath a calibrated burette or chemistry pipetting syringe containing the titrant. Small volumes of the titrant are then added to the analyte and indicator until the indicator changes color in reaction to the titrant saturation threshold, representing arrival at the endpoint of the titration, meaning the amount of titrant balances the amount of analyte present, according to the reaction between the two. Depending on the endpoint desired, single drops or less than a single drop of the titrant can make the difference between a permanent and temporary change in the indicator.

Preparation techniques

Typical titrations require titrant and analyte to be in a liquid (solution) form. Though solids are usually dissolved into an aqueous solution, other solvents such as glacial acetic acid or

ethanol are used for special purposes (as in petrochemistry) which specializes in petroleum. Concentrated analytesare often diluted to improve accuracy.

Many non-acid–base titrations require a constant pH during the reaction. Therefore, a buffer solutionmay be added to the titration chamber to maintain the pH.

In instances where two reactants in a sample may react with the titrant and only one is the desired analyte, a separate masking solution may be added to the reaction chamber which eliminates the effect of the unwanted ion.

Some reduction-oxidation (redox) reactions may require heating the sample solution and titrating while the solution is still hot to increase the reaction rate. For instance, the oxidation of some oxalate solutions requires heating to 60 °C (140 °F) to maintain a reasonable rate of reaction.

Titration curves

A titration curve is a curve in graph the *x*-coordinate of which represents the volume of titrant added since the beginning of the titration, and the *y*-coordinate of which represents the concentration of the analyte at the corresponding stage of the titration (in an acid–base titration, the *y*-coordinate usually represents the pH of the solution).

In an acid–base titration, the titration curve represents the strength of the corresponding acid and base. For a strong acid and a strong base, the curve will be relatively smooth and very steep near the equivalence point. Because of this, a small

change in titrant volume near the equivalence point results in a large pH change and many indicators would be appropriate (for instancelitmus, phenolphthalein or bromothymol blue).

If one reagent is a weak acid or base and the other is a strong acid or base, the titration curve is irregular and the pH shifts less with small additions of titrant near the equivalence point. For example, the titration curve for the titration between oxalic acid (a weak acid) and sodium hydroxide (a strong base) is pictured. The equivalence point occurs between pH 8-10, indicating the solution is basic at the equivalence point and an indicator such as phenolphthalein would be appropriate. Titration curves corresponding to weak bases and strong acids are similarly behaved, with the solution being acidic at the equivalence point and indicators such as methyl orange and bromothymol blue being most appropriate.

Titrations between a weak acid and a weak base have titration curves which are very irregular. Because of this, no definite indicator may be appropriate and a pH meteris often used to monitor the reaction.

The type of function that can be used to describe the curve is termed a sigmoid function.

Redox titration

Redox titrations are based on a reduction-oxidation reaction between an oxidizing agent and a reducing agent. A potentiometer or a redox indicatoris usually used to determine the endpoint of the titration, as when one of the constituents is the oxidizing agent potassium dichromate. The color change of the solution from orange to green is not definite, therefore

an indicator such as sodium diphenylamine is used. Analysis of wines for sulfur dioxide requires iodine as an oxidizing agent. In this case, starch is used as an indicator; a blue starch-iodine complex is formed in the presence of excess iodine, signalling the endpoint.

Some redox titrations do not require an indicator, due to the intense color of the constituents. For instance, in permanganometry a slight persisting pink color signals the endpoint of the titration because of the color of the excess oxidizing agent potassium permanganate. In iodometry, at sufficiently large concentrations, the disappearance of the deep red-brown triiodide ion can itself be used as an endpoint, though at lower concentrations sensitivity is improved by adding starch indicator, which forms an intensely blue complex with triiodide.

- Color of iodometric titration mixture before (left) and after (right) the end point.

Gas phase titration

Gas phase titrations are titrations done in the gas phase, specifically as methods for determining reactive species by reaction with an excess of some other gas, acting as the titrant. In one common gas phase titration, gaseous ozone is titrated with nitrogen oxide according to the reaction

- $O_3 + NO \rightarrow O_2 + NO_2$.

After the reaction is complete, the remaining titrant and product are quantified (e.g., by Fourier transform spectroscopy) (FT-IR); this is used to determine the amount of analyte in the original sample.

Gas phase titration has several advantages over simple spectrophotometry. First, the measurement does not depend on path length, because the same path length is used for the measurement of both the excess titrant and the product. Second, the measurement does not depend on a linear change in absorbance as a function of analyte concentration as defined by the Beer–Lambert law. Third, it is useful for samples containing species which interfere at wavelengths typically used for the analyte.

Complexometric titration

Complexometric titrations rely on the formation of a complex between the analyte and the titrant. In general, they require specialized complexometric indicators that form weak complexes with the analyte.

The most common example is the use of starch indicator to increase the sensitivity of iodometric titration, the dark blue complex of starch with iodine and iodide being more visible than iodine alone. Other complexometric indicators are Eriochrome Black T for the titration of calcium and magnesium ions, and the chelating agentEDTA used to titrate metal ions in solution.

Zeta potential titration

Zeta potential titrations are titrations in which the completion is monitored by the zeta potential, rather than by an indicator, in order to characterize heterogeneous systems, such as colloids. One of the uses is to determine the iso-electric point when surface charge becomes zero, achieved by changing the pH or adding surfactant. Another use is to determine the optimum dose for flocculation or stabilization.

Assay

An assay is a type of biological titration used to determine the concentration of a virus or bacterium. Serial dilutions are performed on a sample in a fixed ratio (such as 1:1, 1:2, 1:4, 1:8, etc.) until the last dilution does not give a positive test for the presence of the virus. The positive or negative value may be determined by inspecting the infected cells visually under a microscope or by an immunoenzymetric method such as enzyme-linked immunosorbent assay (ELISA). This value is known as the titer.

Measuring the endpoint of a titration

Different methods to determine the endpoint include:

- Indicator: A substance that changes color in response to a chemical change. An acid–base indicator (e.g., phenolphthalein) changes color depending on the pH.Redox indicators are also used.

A drop of indicator solution is added to the titration at the beginning; the endpoint has been reached when the color changes.

- Potentiometer: An instrument that measures the electrode potential of the solution. These are used for redox titrations; the potential of the working electrode will suddenly change as the endpoint is reached.
- pH meter: A potentiometer with an electrode whose potential depends on the amount of H ion present in the solution. (This is an example of an ion-selective electrode.) The pH of the solution is measured throughout the titration, more accurately than with an indicator; at the endpoint there will be a sudden change in the measured pH.
- Conductivity: A measurement of ions in a solution. Ion concentration can change significantly in a titration, which changes the conductivity. (For instance, during an acid–base titration, the H and OH ions react to form neutral H_2O.) As total conductance depends on all ions present in the solution and not all ions contribute equally (due to mobility and ionic strength), predicting the change in conductivity is more difficult than measuring it.
- Color change: In some reactions, the solution changes color without any added indicator. This is often seen in redox titrations when the different oxidation states of the product and reactant produce different colors.
- Precipitation: If a reaction produces a solid, a precipitate will form during the titration. A classic example is the reaction between Ag and Cl to form

the insoluble salt AgCl. Cloudy precipitates usually make it difficult to determine the endpoint precisely. To compensate, precipitation titrations often have to be done as "back" titrations (see below).

- Isothermal titration calorimeter: An instrument that measures the heat produced or consumed by the reaction to determine the endpoint. Used in biochemical titrations, such as the determination of how substrates bind to enzymes.
- Thermometric titrimetry: Differentiated from calorimetric titrimetry because the heat of the reaction (as indicated by temperature rise or fall) is not used to determine the amount of analyte in the sample solution. Instead, the endpoint is determined by *the rate of temperature change*.
- Spectroscopy: Used to measure the absorption of light by the solution during titration if the spectrum of the reactant, titrant or product is known. The concentration of the material can be determined by Beer's Law.
- Amperometry: Measures the current produced by the titration reaction as a result of the oxidation or reduction of the analyte. The endpoint is detected as a change in the current. This method is most useful when the excess titrant can be reduced, as in the titration of halides with Ag.

Endpoint and equivalence point

Though the terms equivalence point and endpoint are often used interchangeably, they are different terms. *Equivalence point* is the theoretical completion of the reaction: the volume

of added titrant at which the number of moles of titrant is equal to the number of moles of analyte, or some multiple thereof (as in polyprotic acids). *Endpoint* is what is actually measured, a physical change in the solution as determined by an indicator or an instrument mentioned above.

There is a slight difference between the endpoint and the equivalence point of the titration. This error is referred to as an indicator error, and it is indeterminate.

Back titration

Back titration is a titration done in reverse; instead of titrating the original sample, a known excess of standard reagent is added to the solution, and the excess is titrated. A back titration is useful if the endpoint of the reverse titration is easier to identify than the endpoint of the normal titration, as with precipitation reactions. Back titrations are also useful if the reaction between the analyte and the titrant is very slow, or when the analyte is in a non-soluble solid.

Graphical methods

The titration process creates solutions with compositions ranging from pure acid to pure base. Identifying the pH associated with any stage in the titration process is relatively simple for monoprotic acids and bases. The presence of more than one acid or base group complicates these computations. Graphical methods, such as the equiligraph, have long been used to account for the interaction of coupled equilibria. These graphical solution methods are simple to implement, however they are used infrequently.

Particular uses

Acid–base titrations

- For biodiesel fuel: waste vegetable oil (WVO) must be neutralized before a batch may be processed. A portion of WVO is titrated with a base to determine acidity, so the rest of the batch may be neutralized properly. This removes free fatty acids from the WVO that would normally react to make soap instead of biodiesel fuel.
- Kjeldahl method: a measure of nitrogen content in a sample. Organic nitrogen is digested into ammonia with sulfuric acid and potassium sulfate. Finally, ammonia is back titrated with boric acid and then sodium carbonate.
- Acid value: the mass in milligrams of potassium hydroxide (KOH) required to titrate fully an acid in one gram of sample. An example is the determination of free fatty acid content.
- Saponification value: the mass in milligrams of KOH required to saponify a fatty acid in one gram of sample. Saponification is used to determine average chain length of fatty acids in fat.
- Ester value (or ester index): a calculated index. Ester value = Saponification value – Acid value.
- Amine value: the mass in milligrams of KOH equal to the amine content in one gram of sample.
- Hydroxyl value: the mass in milligrams of KOH corresponding to hydroxyl groups in one gram of

sample. The analyte is acetylated using acetic anhydride then titrated with KOH.

Redox titrations

- Winkler test for dissolved oxygen: Used to determine oxygen concentration in water. Oxygen in water samples is reduced using manganese(II) sulfate, which reacts with potassium iodide to produce iodine. The iodine is released in proportion to the oxygen in the sample, thus the oxygen concentration is determined with a redox titration of iodine with thiosulfate using a starch indicator.
- Vitamin C: Also known as ascorbic acid, vitamin C is a powerful reducing agent. Its concentration can easily be identified when titrated with the blue dye Dichlorophenolindophenol (DCPIP) which becomes colorless when reduced by the vitamin.
- Benedict's reagent: Excess glucose in urine may indicate diabetes in a patient. Benedict's method is the conventional method to quantify glucose in urine using a prepared reagent. During this type of titration, glucose reduces cupric ions to cuprous ions which react with potassium thiocyanate to produce a white precipitate, indicating the endpoint.
- Bromine number: A measure of unsaturation in an analyte, expressed in milligrams of bromine absorbed by 100 grams of sample.
- Iodine number: A measure of unsaturation in an analyte, expressed in grams of iodine absorbed by 100 grams of sample.

Miscellaneous

- Karl Fischer titration: A potentiometric method to analyze trace amounts of water in a substance. A sample is dissolved in methanol, and titrated with Karl Fischer reagent. The reagent contains iodine, which reacts proportionally with water. Thus, the water content can be determined by monitoring the electric potential of excess iodine.

Chapter 4

Sampling

Sub-sampling (chemistry)

In analytical chemistry, **sub-sampling** is a procedure by which a small, representative sample is taken from a larger sample. Good sub-sampling technique becomes important when the large sample is not homogeneous.

Techniques

Coning and quartering

Coning and quartering is a method used by analytical chemists to reduce the sample size of a powder without creating a systematic bias. The technique involves pouring the sample so that it takes on a conical shape, and then flattening it out into a cake. The cake is then divided into quarters; the two quarters which sit opposite one another are discarded, while the other two are combined and constitute the reduced sample. The same process is continued until an appropriate sample size remains. Analyses are made with respect to the sample left behind.

Riffle box splitting

A riffle box is a box containing a number (between 3 and 12) of "chutes" - slotted paths through which particles of the sample may slide. The sample is dropped into the top, and the box

produces two equally divided subsamples. Riffle boxes are commonly used in mining to reduce the size of crushed rock samples prior to assaying.

Concentration

In chemistry, **concentration** is the abundance of a constituent divided by the total volume of a mixture. Several types of mathematical description can be distinguished: mass concentration, molar concentration, number concentration, and volume concentration. A concentration can be any kind of chemical mixture, but most frequently solutes and solvents in solutions. The molar (amount) concentration has variants such as normal concentration and osmotic concentration.

Etymology

The term concentration comes from the word concentrate, from the Frenchconcentrer, from con– + center, meaning “to put at the center”.

Qualitative description

Often in informal, non-technical language, concentration is described in a qualitative way, through the use of adjectives such as "dilute" for solutions of relatively low concentration and "concentrated" for solutions of relatively high concentration. To **concentrate** a solution, one must add more solute (for example, alcohol), or reduce the amount of solvent (for example, water). By contrast, to **dilute** a solution, one

must add more solvent, or reduce the amount of solute. Unless two substances are miscible, there exists a concentration at which no further solute will dissolve in a solution. At this point, the solution is said to be saturated. If additional solute is added to a saturated solution, it will not dissolve, except in certain circumstances, when supersaturation may occur. Instead, phase separation will occur, leading to coexisting phases, either completely separated or mixed as a suspension. The point of saturation depends on many variables such as ambient temperature and the precise chemical nature of the solvent and solute.

Concentrations are often called**levels**, reflecting the mental schema of levels on the vertical axis of a graph, which can be high or low (for example, "high serum levels of bilirubin" are concentrations of bilirubin in the blood serum that are greater than normal).

Related quantities

Several other quantities can be used to describe the composition of a mixture. Note that these should **not** be called concentrations.

Solvation

Solvation (or **dissolution**) describes the interaction of solvent with dissolved molecules. Both ionized and uncharged molecules interact strongly with solvent, and the strength and nature of this interaction influence many properties of the solute, including solubility, reactivity, and color, as well as

influencing the properties of the solvent such as the viscosity and density. In the process of solvation, ions are surrounded by a concentric shell of solvent. Solvation is the process of reorganizing solvent and solute molecules into solvation complexes. Solvation involves bond formation, hydrogen bonding, and van der Waals forces. Solvation of a solute by water is called hydration.

Solubility of solid compounds depends on a competition between lattice energy and solvation, including entropy effects related to changes in the solvent structure.

Distinction from solubility

By an IUPAC definition, solvation is an interaction of a solute with the solvent, which leads to stabilization of the solute species in the solution. In the solvated state, an ion in a solution is surrounded or complexed by solvent molecules. Solvated species can often be described by coordination number, and the complex stability constants. The concept of the solvation interaction can also be applied to an insoluble material, for example, solvation of functional groups on a surface of ion-exchange resin.

Solvation is, in concept, distinct from solubility. Solvation or dissolution is a kinetic process and is quantified by its rate. Solubility quantifies the dynamic equilibrium state achieved when the rate of dissolution equals the rate of precipitation. The consideration of the units makes the distinction clearer. The typical unit for dissolution rate is mol/s. The units for solubility express a concentration: mass per volume (mg/mL), molarity (mol/L), etc.

Solvents and intermolecular interactions

- Solvation involves different types of intermolecular interactions: hydrogen bonding, ion-dipole interactions, and van der Waals forces (which consist of dipole-dipole, dipole-induced dipole, and induced dipole-induced dipole interactions). Which of these forces are at play depends on the molecular structure and properties of the solvent and solute. The similarity or complementary character of these properties between solvent and solute determines how well a solute can be solvated by a particular solvent.

Solvent polarity is the most important factor in determining how well it solvates a particular solute. Polar solvents have molecular dipoles, meaning that part of the solvent molecule has more electron density than another part of the molecule. The part with more electron density will experience a partial negative charge while the part with less electron density will experience a partial positive charge. Polar solvent molecules can solvate polar solutes and ions because they can orient the appropriate partially charged portion of the molecule towards the solute through electrostatic attraction. This stabilizes the system and creates a solvation shell (or hydration shell in the case of water) around each particle of solute. The solvent molecules in the immediate vicinity of a solute particle often have a much different ordering than the rest of the solvent, and this area of differently ordered solvent molecules is called

the cybotactic region. Water is the most common and well-studied polar solvent, but others exist, such as ethanol, methanol, acetone, acetonitrile, and dimethyl sulfoxide. Polar solvents are often found to have a high dielectric constant, although other solvent scales are also used to classify solvent polarity. Polar solvents can be used to dissolve inorganic or ionic compounds such as salts. The conductivity of a solution depends on the solvation of its ions. Nonpolar solvents cannot solvate ions, and ions will be found as ion pairs.

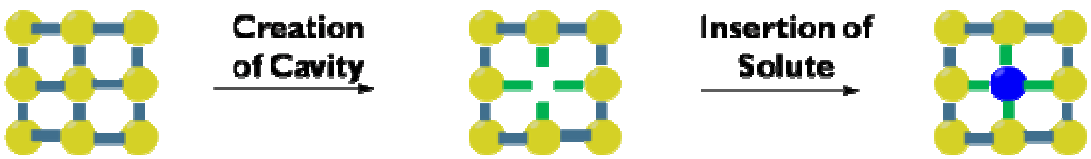
Hydrogen bonding among solvent and solute molecules depends on the ability of each to accept H-bonds, donate H-bonds, or both. Solvents that can donate H-bonds are referred to as protic, while solvents that do not contain a polarized bond to a hydrogen atom and cannot donate a hydrogen bond are called aprotic. H-bond donor ability is classified on a scale (α). Protic solvents can solvate solutes that can accept hydrogen bonds. Similarly, solvents that can accept a hydrogen bond can solvate H-bond-donating solutes. The hydrogen bond acceptor ability of a solvent is classified on a scale (β). Solvents such as water can both donate and accept hydrogen bonds, making them excellent at solvating solutes that can donate or accept (or both) H-bonds.

Some chemical compounds experience solvatochromism, which is a change in color due to solvent polarity. This phenomenon illustrates how different solvents interact differently with the same solute. Other solvent effects include conformational or isomeric preferences and changes in the acidity of a solute.

Solvation energy and thermodynamic considerations

The solvation process will be thermodynamically favored only if the overall Gibbs energy of the solution is decreased, compared to the Gibbs energy of the separated solvent and solid (or gas or liquid). This means that the change in enthalpy minus the change in entropy (multiplied by the absolute temperature) is a negative value, or that the Gibbs energy of the system decreases. A negative Gibbs energy indicates a spontaneous process but does not provide information about the rate of dissolution.

Solvation involves multiple steps with different energy consequences. First, a cavity must form in the solvent to make space for a solute. This is both entropically and enthalpically unfavorable, as solvent ordering increases and solvent-solvent interactions decrease. Stronger interactions among solvent molecules leads to a greater enthalpic penalty for cavity formation. Next, a particle of solute must separate from the bulk. This is enthalpicallyunfavorable since solute-solute interactions decrease, but when the solute particle enters the cavity, the resulting solvent-solute interactions are enthalpically favorable. Finally, as solute mixes into solvent, there is an entropy gain.



The enthalpy of solution is the solution enthalpy minus the enthalpy of the separate systems, whereas the entropy of solution is the corresponding difference in entropy. The solvation energy (change in Gibbs free energy) is the change in enthalpy minus the product of temperature (in Kelvin) times the change in entropy. Gases have a negative entropy of solution, due to the decrease in gaseous volume as gas dissolves. Since their enthalpy of solution does not decrease too much with temperature, and their entropy of solution is negative and does not vary appreciably with temperature, most gases are less soluble at higher temperatures.

Enthalpy of solvation can help explain why solvation occurs with some ionic lattices but not with others. The difference in energy between that which is necessary to release an ion from its lattice and the energy given off when it combines with a solvent molecule is called the enthalpy change of solution. A negative value for the enthalpy change of solution corresponds to an ion that is likely to dissolve, whereas a high positive value means that solvation will not occur. It is possible that an ion will dissolve even if it has a positive enthalpy value. The extra energy required comes from the increase in entropy that results when the ion dissolves. The introduction of entropy makes it harder to determine by calculation alone whether a substance will dissolve or not. A quantitative measure for solvation power of solvents is given by donor numbers.

Although early thinking was that a higher ratio of a cation's ion charge to ionic radius, or the charge density, resulted in more solvation, this does not stand up to scrutiny for ions like iron(III) or lanthanides and actinides, which are readily

hydrolyzed to form insoluble (hydrous) oxides. As these are solids, it is apparent that they are not solvated.

Strong solvent-solute interactions make the process of solvation more favorable. One way to compare how favorable the dissolution of a solute is in different solvents is to consider the free energy of transfer. The free energy of transfer quantifies the free energy difference between dilute solutions of a solute in two different solvents. This value essentially allows for comparison of solvation energies without including solute-solute interactions.

In general, thermodynamic analysis of solutions is done by modeling them as reactions. For example, if you add sodium chloride to water, the salt will dissociate into the ions sodium(+aq) and chloride(-aq). The equilibrium constant for this dissociation can be predicted by the change in Gibbs energy of this reaction.

The Born equationis used to estimate Gibbs free energy of solvation of a gaseous ion.

Recent simulation studies have shown that the variation in solvation energy between the ions and the surrounding water molecules underlies the mechanism of the Hofmeister series.

Macromolecules and assemblies

Solvation (specifically, hydration) is important for many biological structures and processes. For instance, solvation of ions and/or of charged macromolecules, like DNA and proteins, in aqueous solutions influences the formation of heterogeneous

assemblies, which may be responsible for biological function. Another example, protein folding occurs spontaneously, in part because of a favorable change in the interactions between the protein and the surrounding water molecules. Folded proteins are stabilized by 5-10 kcal/mol relative to the unfolded state due to a combination of solvation and the stronger intramolecular interactions in the folded protein structure, including hydrogen bonding. Minimizing the number of hydrophobic side-chains exposed to water by burying them in the center of a folded protein is a driving force related to solvation.

Solvation also affects host–guest complexation. Many host molecules have a hydrophobic pore that readily encapsulates a hydrophobic guest. These interactions can be used in applications such as drug delivery, such that a hydrophobic drug molecule can be delivered in a biological system without needing to covalently modify the drug in order to solubilize it. Binding constants for host–guest complexes depend on the polarity of the solvent.

Hydration affects electronic and vibrational properties of biomolecules.

Filtration

Filtration is a physical or chemical separation process that separates solid matter and fluid from a mixture using a *filter medium* that has a complex structure through which only the fluid can pass. Solid particles that cannot pass through the filter medium are described as *oversize* and the fluid that passes through is called the *filtrate*. Oversize particles may

form a filter cake on top of the filter and may also block the filter lattice, preventing the fluid phase from crossing the filter, known as *blinding*. The size of the largest particles that can successfully pass through a filter is called the effective *pore size* of that filter. The separation of solid and fluid is imperfect; solids will be contaminated with some fluid and filtrate will contain fine particles (depending on the pore size, filter thickness and biological activity). Filtration occurs both in nature and in engineered systems; there are biological, geological, and industrial forms.

Filtration is also used to describe biological and physical systems that not only separate solids from a fluid stream, but also remove chemical species and biological organisms by entrainment, phagocytosis, adsorption and absorption. Examples include slow sand filters and trickling filters. It is also used a general term for microphagy in which organisms use a variety of means to filter small food particles from their environment. Examples range from the microscopic *Vorticella* up to the Basking shark, one of the largest fishes, and the baleen whales, all of which are described as Filter feeders.

Physical processes

- Filtration is used to separate particles and fluid in a suspension, where the fluid can be a liquid, a gas or a supercritical fluid. Depending on the application, either one or both of the components may be isolated.
- Filtration, as a physical operation enables materials of different chemical composition to be separated. A solventis chosen which dissolves one component,

while not dissolving the other. By dissolving the mixture in the chosen solvent, one component will go into the solution and pass through the filter, while the other will be retained.

- Filtration is widely used in chemical engineering. It may be combined with other unit operations to process the feed stream, as in the biofilter, which is a combined filter and biological digestion device.
- Filtration differs from sieving, where separation occurs at a single perforated layer (a sieve). In sieving, particles that are too big to pass through the holes of the sieve are retained (see particle size distribution). In filtration, a multilayer lattice retains those particles that are unable to follow the tortuous channels of the filter. Oversize particles may form a cake layer on top of the filter and may also block the filter lattice, preventing the fluid phase from crossing the filter (blinding). Commercially, the term filter is applied to membranes where the separation lattice is so thin that the surface becomes the main zone of particle separation, even though these products might be described as sieves.
- Filtration differs from adsorption, where separation relies on surface charge. Some adsorption devices containing activated charcoal and ion-exchange resinare commercially called filters, although filtration is not their principal mechanical function.
- Filtration differs from removal of magnetic contaminants from fluids with magnets (typically lubrication oil, coolants and fuel oils), because there is no filter medium. Commercial devices called

'magnetic filters' are sold, but the name reflects their use, not their mode of operation.

- In biological filters, oversize particulates are trapped and ingested and the resulting metabolites may be released. For example, in animals (including humans), renal filtration removes waste from the blood, and in water treatment and sewage treatment, undesirable constituents are removed by adsorption into a biological film grown on or in the filter medium, as in slow sand filtration.

Methods

- There are many different methods of filtration; all aim to attain the separation of substances. Separation is achieved by some form of interaction between the substance or objects to be removed and the filter. The substance that is to pass through the filter must be a fluid, i.e. a liquid or gas. Methods of filtration vary depending on the location of the targeted material, i.e. whether it is dissolved in the fluid phase or suspended as a solid. There are several laboratory filtration techniques depending on the desired outcome namely, hot, cold and vacuum filtration. Some of the major purposes of getting the desired outcome are, for the removal of impurities from a mixture or, for the isolation of solids from a mixture. **Hot filtration** method is mainly used to separate solids from a hot solution. This is done in order to prevent crystal formation in the filter funnel and other apparatuses that comes in contact with the solution. As a result, the apparatus and the

solution used are heated in order to prevent the rapid decrease in temperature which in turn, would lead to the crystallization of the solids in the funnel and hinder the filtration process. One of the most important measures to prevent the formation of crystals in the funnel and to undergo effective hot filtration is the use stemless filter funnel. Due to the absence of a stem in the filter funnel, there is a decrease in the surface area of contact between the solution and the stem of the filter funnel, hence preventing re-crystallization of solid in the funnel, adversely affecting the filtration process.

Cold filtration method is the use of ice bath in order to rapidly cool down the solution to be crystallized rather than leaving it out to cool it down slowly in the room temperature. This technique results to the formation of very small crystals as opposed to getting large crystals by cooling the solution down at room temperature.

Vacuum filtration technique is mostly preferred for small batches of solution in order to quickly dry out small crystals. This method requires a Büchner funnel, filter paper of smaller diameter than the funnel, Büchner flask, and rubber tubing to connect to vacuum source.

Filter media

Filter media are the materials used to do the separation of materials.

Two main types of filter media are employed in laboratories: *surface filters*, which are solid sieves which trap the solid

particles, with or without the aid of filter paper (e.g. Büchner funnel, belt filter, rotary vacuum-drum filter, cross-flow filters, screen filter), and *depth filters*, a bed of granular material which retains the solid particles as they pass (e.g. sand filter). The surface filter type allows the solid particles, i.e. the residue, to be collected intact; the depth filter does not permit this. However, the depth filter is less prone to clogging due to the greater surface area where the particles can be trapped. Also, when the solid particles are very fine, it is often cheaper and easier to discard the contaminated granules than to clean the solid sieve. Filter media can be cleaned by rinsing with solvents or detergents or backwashing. Alternatively, in engineering applications, such as swimming pool water treatment plants, they may be cleaned by backwashing. Self-cleaning screen filters utilize point-of-suction backwashing to clean the screen without interrupting system flow.

Achieving flow through the filter

Fluids flow through a filter due to a difference in pressure—fluid flows from the high-pressure side to the low-pressure side of the filter. The simplest method to achieve this is by gravity and can be seen in the coffeemaker example. In the laboratory, pressure in the form of compressed air on the feed side (or vacuum on the filtrate side) may be applied to make the filtration process faster, though this may lead to clogging or the passage of fine particles. Alternatively, the liquid may flow through the filter by the force exerted by a pump, a method commonly used in industry when a reduced filtration time is important. In this case, the filter need not be mounted vertically.

Filter aid

Certain filter aids may be used to aid filtration. These are often incompressible diatomaceous earth, or kieselguhr, which is composed primarily of silica. Also used are wood cellulose and other inert porous solids such as the cheaper and safer perlite. Activated carbonis often used in industrial applications that require changes in the filtrates properties, such as altering color or odor.

These filter aids can be used in two different ways. They can be used as a precoat before the slurry is filtered. This will prevent gelatinous-type solids from plugging the filter medium and also give a clearer filtrate. They can also be added to the slurry before filtration. This increases the porosity of the cake and reduces resistance of the cake during filtration. In a rotary filter, the filter aid may be applied as a precoat; subsequently, thin slices of this layer are sliced off with the cake.

The use of filter aids is usually limited to cases where the cake is discarded or where the precipitate can be chemically separated from the filter.

Alternatives

Filtration is a more efficient method for the separation of mixtures than decantation, but is much more time-consuming. If very small amounts of solution are involved, most of the solution may be soaked up by the filter medium.

An alternative to filtration is centrifugation—instead of filtering the mixture of solid and liquid particles, the mixture is centrifuged to force the (usually) denser solid to the bottom,

where it often forms a firm cake. The liquid above can then be decanted. This method is especially useful for separating solids which do not filter well, such as gelatinous or fine particles. These solids can clog or pass through the filter, respectively.

Biological filtration

Biological filtration may take place inside an organism, or the biological component may be grown on a medium in the material being filtered. Removal of solids, emulsified components, organic chemicals and ions may be achieved by ingestion and digestion, adsorption or absorption. Because of the complexity of biological interactions, especially in multi-organism communities, it is often not possible to determine which processes are achieving the filtration result. At the molecular level, it may often by individual catalytic enzyme actions within an individual organisms. The waste products of sone organisms may subsequently broken down by other organisms to extract as much energy as possible and in so doing reducing complex organic molecules to very simple inorganic species such as water, carbon dioxide and nitrogen.

Excretion

Inside mammals reptile and birds, the kidneys function by renal filtration in which the glomerulus selectively removes undesirable constituents such as Urea, followed by selective reabsorption of many substances essential for the body to maintain homeostasis. The complete process is termed excretion. Similar but often less complex solutions are

deployed in all animals even the Protozoa where the contractile vacuole provides a similar function.

Biofilms

Biofilms are often complex communities of bacteria, phages, yeasts and often more complex organisms including protozoa, Rotifers and Annelids which form dynamic and complex, frequently gelatinous films on wet substrates. Such biofilms coat the rocks of most rivers and the sea and they provide the key filtration capability of the Schmutzdecke on the surface of slow sand filters and the film on the filter media of trickling filters which are used to create potable water and treat sewage respectively.

Filter feeders

Filter feeders are organisms that obtain their food by filtering their, generally aquatic, environment. Many of the protozoa are filter feeders using a range of adaptations including rigid spikes of protoplasm held in the water flow as in the Suctoria to various arrangements of beating Cillia to direct particles to the mouth including organisms such as *Vorticella* which have a complex ring of cilia which create a vortex in the flow drafting particles into the oral cavity.Similar feeding techniques are used by the Rotifera and the Ectoprocta. Many aquatic arthropods are filter feeders . Some use rhythmical beating of abdominal limbs to create a water current to the mouth whilst the hairs on the legs trap any particle. Other such as some caddis flies spin fine webs in the water flow to trap particles.

Applications and examples

Many filtration processes include more than one filtration mechanism, and particulates are often removed from the fluid first to prevent clogging of downstream elements.

Particulate filtration includes:

- The coffee filter to separate the coffee infusion from the grounds.
- HEPA filters in air conditioning to remove particles from air.
- Belt filters to extract precious metals in mining.
- Vertical plate filter such as those used in Merrill–Crowe process.
- Nutsche filters typically used in pharmaceutical applications or batch processes that need to capture solids.
- Furnaces use filtration to prevent the furnace elements from fouling with particulates.
- Pneumatic conveying systems often employ filtration to stop or slow the flow of material that is transported, through the use of a baghouse.
- In the laboratory, a Büchner funnelis often used, with a filter paper serving as the porous barrier.
- Air filters are commonly used to remove airborne particulate matter in building ventilation systems, combustion engines, and industrial processes.
- Oil filter in automobiles, often as a canister or cartridge.
- Aquarium filter

Adsorption filtration includes:

- Carbon dioxide removal from breathing gas in rebreathers and life-support systems using scrubber filters,
- Activated carbon filters to remove volatile hydrocarbons, odours, and other contaminants from recirculated breathing gas in closed habitats.

Combined applications include:

- Compressed breathing air production, where the air passes through a particulate filter before entering the compressor, which removes particles likely to damage the compressor, followed by droplet separation after post-compression cooling, and final product adsorption filtration to remove gaseous hydrocarbons contaminants and excessive water vapour. In some cases prefilters using adsorption media are used to control carbon dioxide levels, pressure swing adsorption may be used to increase oxygen fraction, and where the risk of carbon monoxide contamination exists, Hopcalitecatalytic converters may be included in the filtration media of the product. All these processes are broadly referred to as aspects of the filtration of the product.
- Potable water treatment using biofilm filtration in slow sand filters.
- Wastewater treatment using biofilm filtration using trickling filters.

Masking agent

A **masking agent** is a reagent used in chemical analysis which reacts with chemical species that may interfere in the analysis.

In sports a **masking agent** is used to hide or prevent detection of a banned substance or illegal drug like anabolic steroids or stimulants. Diuretics are the simplest form of masking agent and work by enhancing water loss via urine excretion and thus diluting the urine, which results in lower concentrations of the banned substance as more of it is being excreted from the body making it more difficult for laboratories to detect.

Powder

A **powder** is a dry, bulk solid composed of many very fine particles that may flow freely when shaken or tilted. Powders are a special sub-class of granular materials, although the terms *powder* and *granular* are sometimes used to distinguish separate classes of material. In particular, *powders* refer to those granular materials that have the finer grain sizes, and that therefore have a greater tendency to form clumps when flowing. *Granulars* refers to the coarser granular materials that do not tend to form clumps except when wet.

Types

Many manufactured goods come in powder form, such as flour, sugar, ground coffee, powdered milk, copy machine toner, gunpowder, cosmetic powders, and some pharmaceuticals. In

nature, dust, fine sand and snow, volcanic ash, and the top layer of the lunar regolith are also examples.

Because of their importance to industry, medicine and earth science, powders have been studied in great detail by chemical engineers, mechanical engineers, chemists, physicists, geologists, and researchers in other disciplines.

Mechanical properties

Typically, a powder can be compacted or loosened into a vastly larger range of bulk densities than can a coarser granular material. When deposited by sprinkling, a powder may be very light and fluffy. When vibrated or compressed it may become very dense and even lose its ability to flow. The bulk density of coarse sand, on the other hand, does not vary over an appreciable range.

The clumping behavior of a powder arises because of the molecular Van der Waals force that causes individual grains to cling to one another. This force is present not just in powders, but in sand and gravel, too. However, in such coarse granular materials the weight and the inertia of the individual grains are much larger than the very weak Van der Waals forces, and therefore the tiny clinging between grains does not have a dominant effect on the bulk behavior of the material. Only when the grains are very small and lightweight does the Van der Waals force become predominant, causing the material to clump like a powder. The cross-oversize between flow conditions and stick conditions can be determined by simple experimentation.

Many other powder behaviors are common to all granular materials. These include segregation, stratification, jamming and unjamming, fragility, loss of kinetic energy, frictional shearing, compaction and Reynolds' dilatancy.

Powder transport

Powders are transported in the atmosphere differently from a coarse granular material. For one thing, tiny particles have little inertia compared to the drag force of the gas that surrounds them, and so they tend to *go with the flow* instead of traveling in straight lines. For this reason, powders may be an inhalation hazard. Larger particles cannot weave through the body's defenses in the nose and sinus, but will strike and stick to the mucous membranes. The body then moves the mucous out of the body to expel the particles. The smaller particles on the other hand can travel all the way to the lungs from which they cannot be expelled. Serious and sometimes fatal diseases such as silicosis are a result from working with certain powders without adequate respiratory protection.

Also, if powder particles are sufficiently small, they may become suspended in the atmosphere for a very long time. Random motion of the air molecules and turbulence provide upward forces that may counteract the downward force of gravity. Coarse granulars, on the other hand, are so heavy that they fall immediately back to the ground. Once disturbed, dust may form huge dust storms that cross continents and oceans before settling back to the surface. This explains why there is relatively little hazardous dust in the natural environment. Once aloft, the dust is very likely to stay aloft until it meets water in the form of rain or a body of water. Then it sticks and

is washed downstream to settle as mud deposits in a quiet lake or sea. When geological changes later re-expose these deposits to the atmosphere, they may have already cemented together to become mudstone, a type of rock. For comparison, the Moon has neither wind nor water, and so its regolith contains dust but no mudstone.

The cohesive forces between the particles tend to resist their becoming airborne, and the motion of wind across the surface is less likely to disturb a low-lying dust particle than a larger sand grain that protrudes higher into the wind. Mechanical agitation such as vehicle traffic, digging or passing herds of animals is more effective than a steady wind at stirring up a powder.

The aerodynamic properties of powders are often used to transport them in industrial applications. Pneumatic conveying is the transport of powders or grains through a pipe by blowing gas. A gas fluidized bed is a container filled with a powder or granular substance that is *fluffed up* by blowing gas upwardly through it. This is used for fluidized bed combustion, chemically reacting the gas with the powder.

Some powders may be dustier than others. The tendency of a powder to generate particles in the air under a given energy input is called "dustiness". It is an important powder property which is relevant to powder aerosolization process. It also has indications for human exposure to aerosolized particles and associated health risks (via skin contacts or inhalation) at workplaces. Various dustiness testing methods have been established in research laboratories, in order to predict powder behaviors during aerosolization. These methods (laboratory

setups) allow application of a wide range of energy inputs to powdered materials, which simulates different real-life scenarios.

Fire dangers of powders

Many common powders made in industry are combustible; particularly metals or organic materials such as flour. Since powders have a very high surface area, they can combust with explosive force once ignited. Facilities such as flour mills can be vulnerable to such explosions without proper dust mitigation efforts.

Some metals become especially dangerous in powdered form, notably titanium.

Comparison with other substances

A paste or gel might become a powder after it has been thoroughly dried, but is not considered a powder when it is wet because it does not flow freely. Substances like dried clay, although dry bulk solids composed of very fine particles, are not powders unless they are crushed because they have too much cohesion between the grains, and therefore they do not flow freely like a powder. A liquid flows differently than a powder, because a liquid cannot resist any shear stress and therefore it cannot reside at a tilted angle without flowing (that is, it has zero *angle of repose.*) A powder on the other hand is a solid, not a liquid, because it may support shear stresses and therefore may display an angle of repose.

Sample preparation

In analytical chemistry, **sample preparation** refers to the ways in which a sample is treated prior to its analyses. Preparation is a very important step in most analytical techniques, because the techniques are often not responsive to the analyte in its in-situ form, or the results are distorted by interfering species. Sample preparation may involve dissolution, extraction, reaction with some chemical species, pulverizing, treatment with a chelating agent (e.g. EDTA), masking, filtering, dilution, sub-sampling or many other techniques. Treatment is done to prepare the sample into a form ready for analysis by specified analytical equipment. Sample preparation could involve: crushing and dissolution, chemical digestion with acid or alkali, sample extraction, sample clean up and sample pre-concentration.

Separation process

A **separation process** is a method that converts a mixture or solution of chemical substances into two or more distinct product mixtures. At least one product mixture of the separation is enriched in one or more of the source mixture's constituents. In some cases, a separation may fully divide the mixture into pure constituents. Separations exploit differences in chemical properties or physical properties (such as size, shape, mass, density, or chemical affinity) between the constituents of a mixture.

Processes are often classified according to the particular differences they use to achieve separation. If no single

difference can be used to accomplish the desired separation, multiple operations can often be combined to achieve the desired end.

With a few exceptions, elements or compounds exist in nature in an impure state. Often these raw materials must go through a separation before they can be put to productive use, making separation techniques essential for the modern industrial economy.

The purpose of separation may be *analytical*, i.e. to identify the size of each fraction of a mixture is attributable to each component without attempting to harvest the fractions. The purpose of a separation may maybe *preparative*, i.e. to "prepare" fractions for input into processes that benefit when components are separated.

Separations may be performed on a small scale, such as in a laboratory for analytical purposes. Separations may also be performed on a large scale, such as in a chemical plant.

Complete and incomplete separation

Some types of separation require complete purification of a certain component. An example is the production of aluminum metal from bauxite ore through electrolysis refining. In contrast, an incomplete separation process may specify an output to consist of a mixture instead of a single pure component. A good example of an incomplete separation technique is oil refining. Crude oil occurs naturally as a mixture of various hydrocarbons and impurities. The refining process splits this mixture into other, more valuable mixtures

such as natural gas, gasoline and chemical feedstocks, none of which are pure substances, but each of which must be separated from the raw crude.

In both cases of complete and incomplete separation, a series of separations may be necessary to obtain the desired end products. In the case of oil refining, crude is subjected to a long series of individual distillation steps, each of which produces a different product or intermediate.

List of separation techniques

- Sponge, adhesion of atoms, ions or molecules of gas, liquid, or dissolved solids to a surface
- Centrifugation and cyclonic separation, separates based on density differences
- Chelation
- Filtration

Chromatography

Chromatography separates dissolved substances by different interaction with (i.e., travel through) a material.

- High-performance liquid chromatography (HPLC)
- Thin-layer chromatography (TLC)
- Countercurrent chromatography (CCC)
- Droplet countercurrent chromatography (DCC)
- Paper chromatography
- Ion chromatography
- Size-exclusion chromatography
- Affinity chromatography

- Centrifugal partition chromatography
- Gas chromatography and Inverse gas chromatography
- Crystallization
- Decantation
- Demister (vapor), removes liquid droplets from gas streams
- Distillation, used for mixtures of liquids with different boiling points
- Drying, removes liquid from a solid by vaporization or evaporation

Electrophoresis

Electrophoresis, separates organic molecules based on their different interaction with a gel under an electric potential (i.e., different travel)

- Capillary electrophoresis
- Electrostatic separation, works on the principle of corona discharge, where two plates are placed close together and high voltage is applied. This high voltage is used to separate the ionized particles.
- Elutriation
- Evaporation

Extraction

- Extraction
- Leaching
- Liquid-liquid extraction
- Solid phase extraction

- Supercritical fluid extraction
- Subcritical fluid extraction
- Field flow fractionation

Flotation

- Flotation
- Dissolved air flotation, removes suspended solids non-selectively from slurry by bubbles that are generated by air coming out of solution
- Froth flotation, recovers valuable, hydrophobic solids by attachment to air bubbles generated by mechanical agitation of an air-slurry mixture, which floats, and are recovered
- Deinking, separating hydrophobic ink particles from the hydrophilic paper pulp in paper recycling
- Flocculation, separates a solid from a liquid in a colloid, by use of a flocculant, which promotes the solid clumping into flocs
- Filtration – Mesh, bag and paper filters are used to remove large particulates suspended in fluids (e.g., fly ash) while membrane processes including microfiltration, ultrafiltration, nanofiltration, reverse osmosis, dialysis (biochemistry)utilisingsynthetic membranes, separates micrometre-sized or smaller species
- Fractional distillation
- Fractional freezing
- Oil-water separation, gravimetrically separates suspended oil droplets from waste water in oil refineries, petrochemical and chemical plants, natural gas processing plants and similar industries

- Magnetic separation
- Precipitation
- Recrystallization
- Scrubbing, separation of particulates (solids) or gases from a gas stream using liquid.
- Sedimentation, separates using vocal density pressure differences
- Gravity separation
- Sieving
- Stripping
- Sublimation
- Vapor-liquid separation, separates by gravity, based on the Souders-Brown equation
- Winnowing
- Zone refining