4. Analytical Chemistry

Measurements of the chemical compositions of materials and the levels of certain substances in them are vital when assessing and improving public health, safety and the environment, are necessary to ensure trade equity, and are required when monitoring and improving industrial products and services. Chemical measurements play a crucial role in most areas of the economy, including healthcare, food and nutrition, agriculture, environmental technologies, chemicals and materials, instrumentation, electronics, forensics, energy, and transportation.

This chapter presents a broad overview of the analytical techniques that can be used to perform the *higher order chemical characterization of materials*. Techniques covered include mass spectrometry, molecular spectrometry, atomic spectrometry, nuclear analytical methods, chromatographic methods and classical chemical methods.

For each technique, information is provided on the principle(s) of operation, the scope of the technique, the nature of the sample that can be used, qualitative analysis, traceable quantitative analysis, and key references. Examples of representative data are provided for each technique, where possible.

4.1 Bulk Chemical Characterization

4.1.1 Mass Spectrometry

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Principles of the Technique. The inductively coupled plasma (ICP) associated with this technique is an atmospheric-pressure argon plasma that is produced in a quartz torch connected to a radiofrequency (RF) generator. Operating RF powers are typically between 1000 and 1500 W. The aqueous liquid sample solution is injected as an aerosol into the axial channel of the ICP, where temperatures on the order of 7000 K prevail. Here the sample is decomposed into its constituent elements, which are ionized. The ions are differentially

pumped into a mass spectrometer (MS) through a sampling cone and skimmer. The relative ion count rates are used to gauge the relative concentrations of the elements in the sample. The most common instruments use quadrupole mass spectrometers with unit mass resolution capabilities. Higher resolution magnetic sector mass spectrometers are also available.

Scope. ICP-MS is used primarily for quantitative elemental analysis, in other words to determine the concentrations of specific elements in a sample. It is also used to measure elemental isotope ratios. It is roughly 1000 times more sensitive than ICP-atomic emission spectrometry (AES), making it very suitable for trace metal analysis at ng/g and even pg/g solution concentrations. It is widely used in the semiconductor industry to test high-purity production chemicals. It is also heavily used for the environmental monitoring of water and soil samples. ICP-MS is used in geological studies, including isotope ratio studies.

Nature of the Sample. In the most common situations, samples are introduced into the ICP as solutions. If the sample being analyzed is a solid, it must first be dissolved. This typically involves dissolving a 0.1 g sample in 100 mL of acidified aqueous solution. Dissolving most samples will require hot concentrated mineral acids.

An alternative sample introduction system uses laser ablation of solid samples to generate an aerosol of sample particles, which can be injected directly into the ICP.

Qualitative Analysis. Specific elements are identified based on the known mass/charge ratios and isotopic patterns of the elements.

Traceable Quantitative Analysis. For systems using solution sample introduction, the ICP-MS instrumentation must be calibrated using solutions containing known concentrations of the analyte elements. Such calibration solutions are available from many commercial suppliers. These solutions are often traceable to one of a set of NIST single-element solution standard reference materials. The analysis of complex samples can be susceptible to matrix effects (the combined effect that all of the species present in the sample have on the mass spectrometric signal of the analyte). This will lead to measurement bias if the calibration solutions are not matched to the matrix of the sample being analyzed. The method of standard additions is an effective strategy for dealing with matrix effects. Use of an internal standard will also reduce susceptibility to matrix interferences.

For high-accuracy work, quantitation can also be accomplished through isotope dilution. In this procedure the isotope ratio of a pair of analyte isotopes is perturbed by spiking the sample with a known amount of a solution that is enriched in the concentration of an isotope of the element that is naturally not very abundant. The degree of perturbation of the isotope ratio depends on the concentration of the analyte element present in the sample before being spiked, and the concentration can be calculated by measuring the perturbed isotope ratio.

Isobaric interferences occur when two or more ionic species with nominally the same mass-to-charge ratio are present in the ICP. The argon plasma is the source of the most severe of these interferences. For example, ^{40}Ar interferes with ^{40}Ca, and $^{40}Ar^{16}O$ interferes with ^{56}Fe in quadrupole systems. The latter example can be resolved with high-resolution systems.

Calibration of systems using sample introduction based on laser ablation is more difficult. The laser ablation process is very matrix-dependent, and calibration requires the use of solid standards that match the characteristics of the sample quite closely.

Certified reference materials are available in a wide variety of sample matrix types from NIST and other sources. These CRMs should be used to validate ICP-MS methods.

Glow Discharge Mass Spectrometry (GD-MS)

Principles of the Technique. A glow discharge is a reduced-pressure, inert gas plasma maintained between two electrodes. Argon is typically used at absolute pressures of a few hundred Pascals or less, and the solid sample serves as the cathode in the circuit. When the discharge is ignited, Ar^+ ions formed in the plasma are accelerated toward the sample surface by means of electrical fields. A substantial fraction of these Ar^+ ions strike the surface of the sample with enough kinetic energy to eject sample atoms from the surface. In this way, the solid sample is directly atomized. Once in the plasma, sputtered atoms may be ionized through collisions with metastable Ar atoms (Penning ionization) or energetic electrons (electron impact ionization). The ions generated are extracted from the plasma and directed into a mass analyzer with one or more detectors. The ions are separated and detected according to the mass-to-charge (m/z) ratios, resulting in a glow discharge mass spectrum.

GD-MS instruments usually employ quadrupole or double-focusing magnetic sector mass analyzers, although other types have also been reported (such as time-of-flight (TOF), ion cyclotron resonance (ICR), and ion traps). To date, there has only been one commercial GD-MS instrument that can truly be said to have been successful in the marketplace. This is the VG-9000 double-focusing glow discharge mass spectrometer (Thermo Elemental, Cambridge, United Kingdom), costing more than $ 500 000 (To describe experimental procedures adequately, it is occasionally necessary to identify commercial products by manufacturer's name or labels. In no instance does such identification imply endorsement by the National Institute of Standards and Technology, nor does it imply that the particular products or equipment are necessarily the best available for that purpose). The number of VG-9000 instruments in use around the world is probably somewhere between 40 and 60. Some other double-focusing instruments, as well as quadrupole instruments, often custom-built by the user, are also employed.

Scope. Glow discharge devices used in GD-MS may be powered by either a direct-current (DC) or radiofrequency (RF) power supply. In DC-GD-MS, the sample must be electrically conductive, since current must pass through it. Therefore, DC-GD-MS is applicable to conductive bulk samples. In principle, the technique is also applicable to samples consisting of conductive layered materials (such as thin films on surfaces). In such a case, registration of ion intensities as surface layers are *sputtered* away results in a *depth profile* of the sample. However, very little work in this area has been reported so far. RF-GD-MS overcomes the restriction to conductive sample types, because in this configuration it is not necessary for net current to flow through the sample. As a result, RF-GD-MS can be applied to electrically insulating bulk solids and coated surfaces with insulating substrates and/or layers.

The greatest strengths of GD-MS, whether DC or RF, are the high sensitivities and low limits of detection available (sub- to low ng/g range directly in the solid state). Owing to these characteristics, the technique has been most widely applied for purity assessments of highly-pure, solid materials, such as those used in the semiconductor industry. The ability to detect H, O and N is also advantageous for this application. Isobaric interferences are not generally problematic in GD-MS, unless low-resolution mass analyzers (such as quadrupoles, which have only unit resolution) are used.

Disadvantages of GD-MS include the fact that matrix effects, while relatively mild compared to many other direct solid techniques, are nonetheless present. Matrix effects manifest themselves mainly as overall sputtering rates that vary from matrix to matrix. Fortunately, even though this is true, once sputtering has reached *steady state* for a given sample, the relative fluxes of sputtered atoms accurately reflects the stoichiometry of that sample. Another disadvantage of GD-MS is that it is inherently a *relative* technique, requiring reference materials for calibration. Unfortunately, reference materials with certified values in the mass fraction range of greatest interest (< 1 mg/g) are rare. As a result, GD-MS calibration can only be accomplished to the degree allowed by available reference materials.

Nature of the Sample. As noted above, samples amenable to GD-MS analysis include bulk solids and layered surfaces. Bulk solids are often machined into pins for analysis, although flat samples can also be employed.

Qualitative Analysis. Calibration of a GD-MS instrument consists of measuring relative sensitivity factors (RSFs) for the analytes. RSFs are generated by measuring the signal levels for suitable reference materials. When suitable reference materials are lacking, GD-MS cannot be calibrated, resulting in qualitative analysis. Nonetheless, given the very low limits of detection, the technique finds much use in this mode.

Traceable Quantitative Analysis. Quantitative GD-MS analysis (with accuracies in the range of $\pm 30\%$ relative, for example) is performed by employing RSFs generated from matrix-matched reference materials. If RSFs must be developed from non-matrix-matched reference materials, then GD-MS becomes a semiquantitative technique (giving accuracies within a factor of 2 for example). In either case, the calibrants constitute the traceability link to the SI.

Several review articles [4.1–8] are available.

4.1.2 Molecular Spectrometry

Traceability in UV/Vis Molecular Absorption Spectrometry

Principles of the Technique. Molecular absorption spectrometry in the ultraviolet and visible spectral region, often simply known as *UV/Vis*, is one of the oldest and most mature instrumental methods of chem-

ical analysis, with the development of photoelectric detection spectrophotometers dating from the 1920s. Numerous books and review articles have been written on the instrumentation and applications of this mature technology. The present treatment will thus only touch on the bare essentials of the technology as they relate to the subject of assuring traceable measurements at levels of measurement uncertainty that are appropriate to the application, or *fit for purpose*.

The basic measurement of molecular absorption spectrometry is the *absorbance (A)* of a sample, defined as

$$A(\lambda) = \log \frac{I_0(\lambda)}{I(\lambda)} , \tag{4.1}$$

where the numerator of the logarithmic argument is the intensity of light incident on the sample centered at a given wavelength (λ), and the denominator is the reduced intensity of the same distribution as it exits the far side of the absorbing sample. In chemical applications, the sample would normally be a solution containing one or more species that absorb UV/Vis radiation, and the intensity measurements may be made in such a way as to compensate for reflective losses at the faces of the cell containing the solution.

Scope. A plot of A as a function of λ for a known concentration of a neat compound in a transparent solvent, the *absorbance spectrum*, is characteristic of the compound (and sometimes the solvent), and may be employed for qualitative and quantitative analysis. Compounds are frequently characterized by the wavelength and *absorptivity* (absorbance per unit concentration and path length) of the major absorbance bands in the spectrum. Mixtures of absorbing compounds are subject to spectral interference errors during quantitation, since the absorbance bands tend to be relatively broad. Separations and other chemical methods used to avoid these errors are beyond the scope of this article.

Nature of the Sample. The main sample requirement for UV/Vis analyses is at least partial transparency to optical radiation in the range between 200 and 700 nm. Homogeneous, clear solutions are the most usual sample formats for UV/Vis analyses, but there is no requirement that such solutions be liquid, as glasses, polymer mixtures and thin film materials on transparent substrates are also commonly measured. Samples containing dispersed particulates are troublesome for UV/Vis measurements due to photometric errors generated by reflectance and scattering of the incident light by the particles.

Qualitative Analysis. UV/Vis can be used qualitatively to determine the presence or absence of components in a sample; however, the broad absorbance bands of most samples often limit the utility of this approach. The UV/Vis spectrum of a material combined with its luminescence and reflectance spectra determine the color appearance of the material. This aspect of optical spectroscopy, often referred to as color science, has tremendous commercial utility and implications and has evolved into a nearly separate field from optical spectrometry, which is primarily devoted to the quantitation of materials based on their optical spectra.

Traceable Quantitative Analysis. Most absorbance standards and many UV/Vis *methods* specify wavelength accuracy on the order of ± 1 nm. Rare earth elements in solution or in glass provide sharp absorbance bands appropriate to wavelength standardization, and these are available in a sample geometry that is compatible with the most common chemical sample holder. Traceability of these standards to the meter may derive from the intrinsic properties of the absorbing species in the stated matrix, documented along with all the required uncertainty analysis, or by individual or batch calibration or certification of the artifact by a standards-producing organization.

Because the absorbance scale is based on the ratio of two intensities (4.1), accuracy requires that the combination of the wavelength-selection device and the photoelectric detector yield a signal S that is directly proportional to the light intensity I. Instead, allowing for a background B and an intensity-dependent proportionality between signal and intensity $k(I)$ we may have

$$\frac{S_0}{S} = \frac{k(I_0)I_0 + B}{k(I)I + B} . \tag{4.2}$$

Equation (4.2) may be seen to reduce to the desired ratio I_0/I in the special case for which there is no dark current or stray radiant energy (SRE) ($B = 0$) and for which the proportionality between the light intensity and the detector signal is constant $k(I) = k(I_0) = \text{constant}$. Most research-grade spectrophotometers are designed to render these bias sources negligible over the useful analytical range $0 \le A \le 3$, and are frequently employed as *transfer spectrophotometers* to provide absorbance calibrations or absorbance-certified reference materials with traceability to a *reference spectropho-*

tometer maintained by a National Metrology Institute (NMI) such as the National Institute of Standards and Technology (NIST) in the USA. Utility instruments used for routine measurement may also exhibit negligible bias, but calibrated or certified artifact standards are typically run periodically to verify the accuracy of the instrument and to provide a record for quality management or regulatory purposes.

An extensive treatment of the history and development of reference materials for spectrophotometry, as well as current recommendations, has been given in the 50th anniversary publication of the UV Spectrometry Group (UVSG) of the United Kingdom. While NMIs may be credited with designing and distributing spectrophotometric certified reference materials (CRMs) for many years, current trends in fit-for-purpose quality management and multinational commerce favor a more distributed model. The infrastructure for leveraging the production of CRMs into the private sector is supported by the concept of *traceability* as defined by the International Organization for Standardization (ISO) and endorsed by NIST and other NMIs. Producers of CRMs may assert traceability by rigorous documentation of adherence to the requirement of *an unbroken chain of comparisons, all having stated uncertainties* relating the certified values to *stated references*.

ISO Guide 34 provides a detailed framework for the production of CRMs. The accreditation of a laboratory to this guide for the production of specified CRMs is recognized by ISO as a necessary and sufficient condition for the assertion of traceability of the certified values for those materials. Similarly, ISO Guide 17025 confers the mantle of traceability on absorbance and wavelength calibration measurements on customer-furnished artifact samples.

Fluorescence/Phosphorescence Spectroscopy

Principles of the Technique. Fluorescence/phosphorescence spectroscopy is the study of UV, visible and near infrared (NIR) light that is emitted by a chemical species after it has absorbed light. This absorption of light puts the species into an electronically excited state. The emission wavelengths are usually longer or less energetic than the excitation wavelength. A fluorescence spectrometer or fluorometer is the instrument that is used to measure the intensity of emitted light, or emission, as a function of wavelength. Fluorescence and phosphorescence are the two types of emission activated by light absorption. Fluorescence has a short lifetime (typically 1–10 ns) and usually arises from an *allowed* transition from an excited singlet state to the ground electronic state. Phosphorescence has a long lifetime (typically 1 ms–1 s) and usually arises from a *forbidden* transition from an excited triplet state to the ground electronic state. Emission with lifetimes that fall in the range from hundreds of nanoseconds to hundreds of microseconds is not uncommon and may possess characteristics of both fluorescence and phosphorescence. Fluorescence is almost always more intense than phosphorescence, under similar conditions. For the sake of simplicity and space, fluorescence detection will be discussed exclusively in what follows, but much of this discussion also applies to phosphorescence.

Scope and Qualitative Analysis. Fluorescence spectroscopy is a *background-free* technique, meaning that the measured signal should be equal to zero (the lower bound for the signal) if the sample does not fluoresce. The upper bound for the signal is not well-defined, making it very difficult to express fluorescence intensity on a scale that is independent of instrument geometry and excitation intensity (fluorescence intensity is dependent upon the excitation intensity, the absorption coefficient, concentration, quantum yield, and geometry of the sample, the instrument geometry, and the response of the detection system). This is quite different from absorption spectrophotometry, for instance, where the transmittance is measured as the ratio of the light intensity transmitted by the sample versus the light intensity incident upon the sample, the latter intensity being the upper limit. Because of this difficulty in defining the fluorescence intensity scale, few have attempted to compare fluorescence intensities measured by different instruments or even by the same instrument over long periods of time. For most of its history, fluorescence spectroscopy has mainly been used to determine whether or not a particular species is present in an unknown sample. When this technique has been used to quantitate amounts of analyte, it has been done using a calibration curve of fluorescence intensity versus concentration, which is only useful for measuring the particular analyte on the particular instrument on which the curve was obtained. Only in recent years has the need to compare fluorescence intensities and spectral contours taken at different times or on different instruments become crucial in order to ensure compliance with quality and regulatory standards in many key areas where fluorescence detection is used.

Fluorescent species have been detected at low concentrations since the first commercial fluorometers were produced in the 1950s. The high sensitivity of fluorescence detection enables nanomolar concen-

trations of fluorophores to be detected routinely and single molecules to be detected with more difficulty. In the past, fluorometry was primarily viewed as a research technique due to the fact that few naturally occurring compounds fluoresce. This view has changed dramatically over the past 10–15 years due to the synthesis of a long list of fluorescent probes, which have been tailored to bond to an equally large and varied number of analytes. This development has caused fluorescence to become a highly selective detection technique, thereby expanding its scope of use into a broad range of commercial applications. The powerful combination of sensitivity and selectivity of fluorescence-based measurements has fueled the dramatic growth in biotechnology and drug discovery in recent years. Fluorescence detection was used to map the human genome, and is being used to map proteins and the genes of viruses and living organisms. It is also being used by pharmaceutical companies to single out promising drug *leads* in combinatorial chemistry libraries of compounds using microwell plates and microarrays, and in clinical diagnostics to count relative populations of diseased or drug-responsive cells using flow cytometry. In addition, fluorescence detection has important applications in environmental monitoring, where it is used to detect contaminants such as polycyclic aromatic hydrocarbons (PAHs).

Nature of the Sample. The conventional fluorometer is a benchtop instrument with a light source (such as a Xe lamp) and wavelength selector for excitation before the sample, a sample compartment with a mount to hold the sample in the path of the excitation light, and a wavelength selector and detector for emission after the sample. The wavelength selector is usually a monochromator with a range from 200 to 800 nm. Fluorescence from liquid samples is measured at a 90° angle relative to the excitation beam using a rectangular cuvette that holds 3 to 4 mL. Fluorescence from solid samples is typically collected from the same side of the sample as the excitation beam at an angle of 90° or less with respect to the excitation beam. Portable fluorometers are becoming more popular due to their low cost and ease of use in the field. They commonly use a grating to disperse fluorescence onto a linear, charge-coupled device (CCD) array detector, and a fiber optic is often used to both deliver excitation light to and collect fluorescence from the sample. Portable fluorometers have a broad emission range from 200 to 1100 nm, but use a lamp, light-emitting diode (LED) or diode laser without a wavelength selection device for excitation. They are less sensitive and have lower spectral resolutions than benchtop instruments. High-throughput fluorometers – microwell and microarray readers – are designed to measure a large number of samples in a short period of time. Typical sample densities are 1536 wells on a 12.8 cm (L) × 8.5 cm (W) microwell plate and 40 000 spots on a 7.5 cm (L) × 2.5 cm (W) microarray chip. A lamp with a bandpass filter and a monochromatic laser are used for excitation with microwell plate and microarray readers, respectively. Filters with a bandpass of 25 nm or more are used for wavelength selection of emission.

Traceable Quantitative Analysis. Quantitative determinations of fluorescent or fluorescently labeled species are performed most often by first making a plot of fluorescence intensity versus concentration using standard solutions of the species of interest. This plot is typically linear at low concentrations, so it can be fitted to a straight line function. The fitted function can then be used to determine the concentration of an unknown solution by measuring its fluorescence intensity, but a calibration curve of this type is only useful for the instrument on which it was obtained with a fixed set of instrument parameters and for that one particular species used to obtain the curve. In addition, organic dyes, used to make standard solutions, are difficult to acquire with known purity, and their fluorescence intensity is often environment-dependent, making them less than ideal standards for quantifying fluorescent probe concentrations. As fluorescence-based assays become more and more quantitative, particularly in regulated areas such as clinical diagnostics and pharmaceutical quality assurance, it is essential for fluorescence intensities to be comparable between different instruments and laboratories. This requires fluorometers to be rigorously calibrated.

Fluorometers with a wavelength selection device, such as a monochromator, should first be calibrated for wavelength and bandwidth accuracy for emission and excitation. Then, the fluorescence intensity should be calibrated as a function of emission and/or excitation wavelength, depending on whether the intensity is being measured as a function of one or both wavelength axes. Wavelength and bandwidth accuracy are most commonly calibrated using atomic lamps at the sample and/or source positions. The Raman line of water with a Stokes shift of 3382 cm^{-1} can also be used to calibrate one wavelength axis if the other has already been calibrated.

The fluorescence intensity as a function of wavelength, or the spectral emissivity, can be calibrated relatively (calibrated for spectral shape) or absolutely (calibrated for both shape and absolute intensity). Relative intensity can be calibrated as a function of emission or excitation wavelength using a calibrated light source or a calibrated detector at the sample position, respectively. An absolute calibration is usually done in one of two ways: either by using a calibrated detector at the sample position followed by a calibrated reflector at the sample position, or by using a calibrated light source at the sample position followed by a calibrated reflector at the sample position. The absolute calibration yields a correction factor for intensity as a function of wavelength that enables the bispectral luminescence radiance factor (BLRF) of a fluorescent sample to be determined from its measured fluorescence spectrum. The BLRF is defined as the ratio of the radiance of the fluorescence of a sample versus the excitation irradiance incident on the sample, and is a function of both the excitation and emission wavelengths. A fluorescence spectrum or intensity that is expressed in BLRFs is termed *absolute* because it is not instrument-dependent, only sample-dependent. Material standards supplied with certified values for relative intensity, or better still BLRFs, would greatly simplify the calibration process, enabling non-experts to calibrate their fluorometers with greater ease and at less expense. Several national metrology institutes are developing certified reference materials of this type, but at present, SRM 936a quinine sulfate dihydrate, a relative intensity standard that effectively covers the emission region from 390 to 590 nm, is the only such standard that is commercially available.

Raman Spectroscopy

Principles of the Technique. The Raman effect occurs when a sample is irradiated with intense monochromatic light, usually from a laser. The resulting inelastically scattered light is shifted in frequency due to interactions with the vibrational modes of the chemical sample. Although similar in principle to infrared spectroscopy, quantum mechanical selection rules choose only those vibrational modes that cause the polarizability of the chemical bond to become Raman active. The effectiveness of the bond toward scattering is directly dependent upon the polarizability; polarizability decreases as the electron density increases or the length of the bond decreases. As a result, symmetric vibrational bond stretches are typically Raman active.

The sample to be measured is illuminated with a laser and the resulting scattered light measured with a spectrometer. Raman spectrometers can be either grating or Fourier transform instruments. Because the scattering is very inefficient, typically less than one photon in 10^6, very efficient filters are required to separate the laser scatter from the Raman spectrum. The invention of holographic notch filters allowed rejection ratios as great as 10^{12} and enabled the use of optically efficient spectrometers coupled with high quantum efficiency detectors. The net result is that Raman spectroscopy, once a strictly academic technique requiring room-filling instrumentation, is now a real-time, suitcase-portable analytical technique.

Scope and Nature of the Sample. Raman spectroscopy is applicable to gases, liquid, and solid samples, and instruments accommodating microsamples as well as bulk chemical samples have been developed. Because the lasers typically used are visible and diode-based, fiber optic sampling is becoming more common and this is facilitating its use in routine qualitative spectroscopy in nontraditional locations. An example is its use on loading docks at pharmaceutical companies to assure the identity of incoming materials. Raman spectroscopy is predominantly a qualitative technique. That is, many applications capitalize on the unique spectrum that each compound or class of compounds exhibits. It is suitable for the identification of both organic and inorganic materials.

Raman spectroscopy's strengths are that it is nondestructive and requires very minimal, if any, sample preparation. It is also rapid, as the spectra of neat compounds can be acquired in seconds. Water has very weak Raman scatter. Aqueous samples are measurable using Raman in contrast to IR spectroscopy, where water is a major interference. An additional advantage of the technique (over IR) is that very low wavenumber vibrational modes are easily measured. Typical commercial analytical systems allow measurement to 150 cm^{-1}, and some as low as 4 cm^{-1}. IR, in contrast, is limited to at best 500 cm^{-1}. The low-frequency region provides detailed information about crystal structure and is becoming commonly used in the pharmaceutical industry for polymorph characterization.

Raman spectroscopy's weakness is that it is an emission measurement (single beam), and as a result the spectra are highly instrument-dependent. The resulting spectrum is a convolution of the instrument response (due to laser color, optical geometry, fiber optic sampling, and other factors), the scattering characteristics of the sample, and the *true* Raman spectrum of the compound. Because of the complexities of this convolution,

all libraries of Raman spectra today are instrument-dependent. Unfortunately, interpreting Raman spectra from first principles is often difficult for the nonspecialist. In addition, Raman spectroscopy is not a trace quantitative analytical technique. For even strongly scattering samples, such as benzene, quantitation of less than 1% by mass of an analyte is difficult. This is due to the noise characteristics of the measurement and also interference. If a sample or its matrix should fluoresce, this *noise* is several orders of magnitude greater than the Raman signal and may make the measurement impossible. This can be alleviated, in part, by switching to longer wavelength lasers, but at the expense of sensitivity. It should be noted though that microscope-based Raman techniques allow the identification of very small quantities of neat materials. Nanogram quantities of organics can be routinely measured, and submonolayer coverage of chemical vapor deposited (CVD) diamond on silicon is a routine measurement for semiconductor manufacturers.

Qualitative Analysis. Raman spectroscopy is primarily a qualitative technique, useful for identifying compounds by their unique spectral features. Unlike infrared spectroscopy, not many Raman libraries exist due to the instrument-dependent response mentioned previously. Those that have been developed are typically useable at a single excitation wavelength, which to date are predominately Fourier transform (FT) Raman systems operating at 1064 nm. NIST is producing a set of artifact standards that will correct the relative intensity axis of Raman spectrometers operating with a variety of commercially important laser excitation wavelengths. This will enable the production of instrument-corrected libraries, allowing their use on differing systems.

Traceable Quantitative Analysis. Quantitative analysis by Raman spectroscopy is a growing practice, but still rare compared to its use for qualitative analysis. Similar to absorbance-based techniques, the Raman emission scattering from a vibrational mode is proportional to the concentration of the analyte. Standard addition and internal standard techniques have been used for quantitative analysis. Recently, however, the use of multivariate statistical techniques is becoming more common for quantitative work with Raman. This method uses a series of spectra of known analyte concentrations to *train* a calibration model. The spectrum of an unknown sample is then compared to the training set utilizing a variety of regression-based statistical techniques. Because the full spectrum of the analyte may be used, high precision – and in some cases lower limits of quantitation – may be obtained with these methods than with similar univariate (Beer's law) type calibrations.

Example Spectrum. A Raman spectrum of cyclohexane is shown in Fig. 4.1. The x-axis is in Raman shift units, expressed as the difference between the absolute wavenumber frequency of the band and the excitation laser. This practice is observed to make the spectra appear similar to infrared spectra. The upper spectrum was acquired using 785 nm laser excitation and is uncorrected for instrument response. The lower spectrum is corrected for instrument response. As can be seen, the C–H stretching region (3300 to 2600 cm^{-1}) is greatly affected by the system response.

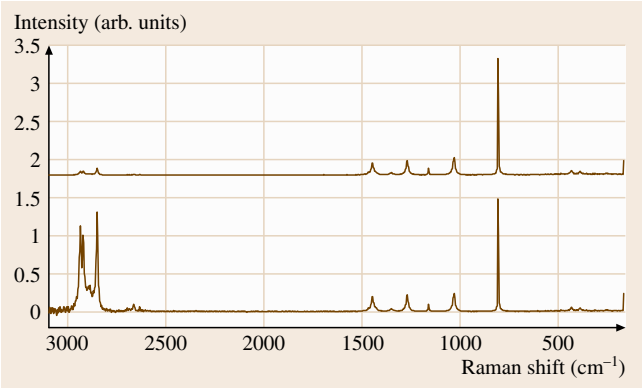

Fig. 4.1 Raman spectroscopy. *Upper*: Raman spectrum of cyclohexane uncorrected for instrument response; *lower*: Raman spectrum of cyclohexane corrected for instrument response

Infrared Spectroscopy

Principles of the Technique. The infrared (IR) region of the spectrum spans from approximately 750 nm to 300 μm, or in wavenumbers roughly from 13 000 to 33 cm^{-1}. Radiation is absorbed in this spectral region when its frequency matches a vibrational mode of the chemical sample. Quantum mechanical selection rules dictate that only those modes of the molecule that undergo a change in dipole moment will absorb in the infrared. Vibrational modes of homonuclear and/or linear molecules such as Cl_2 or N_2 do not show a net change in charge distribution and thus are not IR-absorbing. Molecules that contain heteroatomic substituents or have nonsymmetric vibrational modes are IR active due to the net charge density change. Carbonyl groups are an example.

In IR spectroscopy the sample to be measured is illuminated with a broadband IR source. The resulting IR spectrum is measured with an IR spectrometer. These spectrometers may be either grating-based or Fourier transform (FT) instruments, although in the last ten years FT instruments have all but replaced the dispersive systems due to their many advantages (speed, sensitivity, signal-to-noise ratio, and cost). As the IR range spans a very large wavelength/wavenumber range, material constraints of the optics comprising the spectrometer, such as the beam splitter, detector sensitivity, and so on, limit the range of typical IR spectrometers from 6000 to 660 cm^{-1}. This is not viewed as a major limitation, as this range covers the entire fundamental vibrational mode space for most organic molecules.

Scope and Nature of the Sample. Infrared spectroscopy is applicable to gases, liquids and solid samples. Instruments accommodating microsamples as well as bulk chemical samples have been developed. With the advent of 2-D focal plane detectors, chemical mapping is becoming more widespread. Compositional mapping has applications in analytical chemistry and in health-related areas such as pathology and forensics.

The technique's strengths are many. It is highly sensitive due to the nature of the absorbance, and microgram quantities of neat material are readily measured with excellent sensitivity. The instrumentation is relatively inexpensive and easily used by the nonspecialist. Because the technique is absorbance-based (based on a transmittance ratio), all spectra look alike on all spectrometers, unlike emission techniques such as Raman spectroscopy. As a result, a large number of spectral libraries exist. Specialized IR libraries include polymers, pharmaceuticals, forensic materials, drugs, explosives, pesticides, chemical warfare agents – the list is essentially endless. In fact, several commercial libraries comprise more than 220 000 spectra. Arguably, IR is the second most useful identification technique behind mass spectrometry, with much less sample preparation required. It is, in principle, nondestructive to the sample, and has been paired with other analytical techniques to provide additional information. Examples include gas chromatography-infrared (GC-IR) and thermal gravimetric analysis-infrared (TGA-IR).

The weakness of IR spectroscopy is in fact due to the high absorptance of the vibrational modes of chemical bonds. Neat compounds will typically completely absorb portions of the IR spectrum unless the optical pathlength through the sample is kept below 100 μm. This requires the use of expensive and difficult-to-maintain optical cells, the materials of which are limited to inorganic salts (such as KBr or NaCl) or expensive semiconductor-type materials. Glass or quartz – commonly used for UV/Vis, Raman or fluorescence spectroscopy – are not appropriate as they completely absorb all IR radiation. In addition, water cannot be used as a solvent for similar reasons. Water vapor can be a significant interference, which requires a dry nitrogen purge of the spectrometer. All the above require significant time for sample preparation, and significant skill may be required of the operator.

Qualitative Analysis. IR is most commonly used to identify chemical samples, as the IR spectrum of a compound represents one of its truly unique physical properties. No two compounds have identical IR spectra, with the exception of optical isomers. As mentioned, sample dilution/preparation can be difficult for traditional transmission measurements. However, an array of new sampling techniques enable acquisition of the IR spectra of intact samples. Attenuated total reflectance utilizes the principle of frustrated total internal reflectance to control the depth of penetration into sample. One micrometer sample pathlengths are easily achieved and highly scattering/absorbing samples such as cloth, oil or even foods such as peanut butter are readily measured. New polytetrafluoroethylene (PTFE, teflon) polymer cards allow liquid samples to be smeared onto disposable cards for transmission measurements, eliminating the need for expensive and delicate salt windows.

Traceable Quantitative Analysis. Infrared spectroscopy may be used for quantitative analysis, but it is only infrequently used in this way compared to UV/Vis transmission measurements due to the difficulty in maintaining similar cell pathlengths for the sample and reference cells. The tolerance required would be on the order of 1 μm or less. When IR is used for quantitative analysis, typically the internal standard or standard addition methods are used to relate the sample concentration to the band area (or height). NIST produces one SRM appropriate to IR spectrometry. SRM 1921 is a wavelength calibration standard useful for both dispersive and FT instruments.

Example Spectrum. An example infrared spectrum of benzene is displayed in Fig. 4.2.

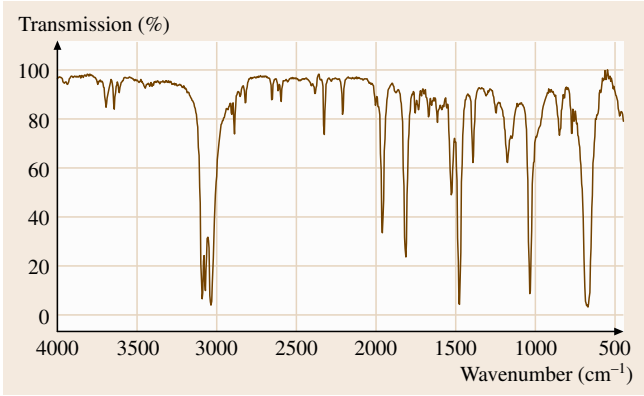

Fig. 4.2 Infrared spectroscopy: IR spectrum of benzene

Quantitative Proton Nuclear Magnetic Resonance Spectroscopy – ^{1}H QNMR

Principles of the Technique. When a nucleus with a nonzero spin quantum number is placed in a homogeneous external magnetic field, the spin of the nuclear charge creates a magnetic dipole that aligns itself in specific orientations with the external field. The quantum spin number of the nucleus (I) determines the number of these orientations ($2I + 1$), and the energy difference between them is $\boldsymbol{B}_0 h\gamma/2\pi$, where $\boldsymbol{B}_0$ is the bulk magnetic field strength, h is Planck's constant, and γ is the magnetogyric ratio, a fundamental constant for each type of magnetically active nucleus. For protons (^{1}H), $I = \frac{1}{2}$, so there are two states with a slight population excess in the lower state. Transitions between the lower energy state and higher level state can be effected by applying radiofrequency radiation (RF) at the Larmor or resonance frequency equal to $\boldsymbol{B}_0\gamma/\pi$. At resonance, the nuclei undergoing the transition absorb energy from the RF that is detected and measured. The RF can be introduced either by continuous wave (CW) scanning (holding the bulk field constant and scanning the RF or vice versa) or by applying an RF pulse. At resonance, the magnetic dipoles not only transit from the low to the higher energy state, but also the phases of their spins synchronize (phase-up) to establish a net magnetization. When the resonance condition is removed, the nuclear spin system reverts to its thermodynamically normal state by first-order rate processes. The means by which the energy state population distribution is re-established is called the spin–lattice relaxation, with a rate constant of $1/T_1$, while the spin dephasing mechanism is called spin–spin relaxation, with a rate constant of $1/T_2$.

The magnetic environments of each of the same nuclei in a molecule are often slightly different depending on the molecular and electronic configurations, and this gives rise to slightly different resonance frequencies and forms the basis for a chemical shift scale that is very useful for identifying the structural environments of atoms in the molecule. Furthermore, the magnetic environment of a given nucleus can be influenced by that of other nearby nuclei, causing a further splitting of the energy levels depending on whether the adjacent dipoles are aligned or opposed. These splitting patterns (or couplings) are used to determine the number and type of the nearest neighbor nuclei in a molecule and their orientations. The resonance frequencies for different types of nuclei are often widely separated (megahertz apart), whereas the chemical shift range for a given nucleus is smaller (kilohertz), and the splitting differences are smaller still (hertz).

Modern NMR spectrometers use superconducting solenoids to provide the bulk magnetic field. While these instruments could be operated in the CW mode, it has proved much more efficient to operate them where the RF is provided through a brief (μs) RF pulse. This excites all of the nuclei simultaneously, and the resulting signals (the free-induction decay or FID) are collected digitally in the time domain (intensity versus time) and then converted to the frequency domain (intensity versus frequency) by Fourier transformation. The signal-to-noise ratios (S/N) of such measurements are improved by collecting and time-averaging multiple FIDs prior to Fourier transformation. The S/N improves by the square root of the number of FIDs collected, provided all of the nuclei have fully relaxed between pulses.

Scope. While there is a wide range of NMR-active nuclei, only a handful are commonly used. Fortunately, isotopes of the most common atoms in organic molecules are in this category. ^{1}H is the most commonly observed nucleus due to its large natural abundance and high relative sensitivity. Other commonly utilized nuclei are shown in Table 4.1.

Nature of the Sample. High-resolution NMR spectra are most commonly collected from liquid samples or materials in solution. Sample sizes range from micrograms to hundreds of milligrams. NMR spectra of solids can be obtained. However, special techniques involving spinning the sample very rapidly about an axis specifically oriented to the bulk field (at the *magic angle*) are necessary to narrow the lineshapes. The NMR process is nondestructive, so samples can be recovered. To avoid interferences, solvents devoid of the nuclei being ob-

Table 4.1 Properties of commonly observed NMR-active nuclei

Nucleus	Resonance at 14.09 T (MHz)	Spin number (I)	Natural abundance (%)	Relative sensitivity	Abundance × sensitivity
^{1}H	599.944	1/2	99.984	1.000	99.984
^{19}F	564.511	1/2	100	0.834	83.4
^{31}P	242.862	1/2	100	0.0664	6.64
^{13}C	150.856	1/2	1.108	0.0159	0.018
^{29}Si	119.192	−1/2	4.7	0.00785	0.037
^{2}H	92.095	1	0.0156	0.00964	0.00015
^{15}N	60.815	−1/2	0.365	0.00104	0.00038

served are often employed. For ^{1}H NMR, deuterated solvents are commonly used. The NMR signals from the deuterium nuclei are also utilized in a separate detection channel to provide a field-frequency lock to stabilize the spectrometer, as it is necessary to maintain the field-frequency ratio to better than 1 part in 10^{9} for modern instruments. Commonly, a small amount of a standard is added along with the solvent to provide a chemical shift reference. Tetramethylsilane (TMS) is often used for this purpose in ^{1}H, ^{13}C and ^{29}Si spectra.

Qualitative Analysis. The chemical shift differences between the various nuclei and the internuclear couplings in a molecule can be used to determine the atomic interconnections in a molecule. The number and chemical type of the observed nuclei can also be determined. Often, only a single structural configuration will fit the spectrum, making it easy to differentiate isomers. Molecular conformations and components of mixtures can also be determined. Even enantiomers can be determined if the molecule of interest interacts with a chiral solvent or another material to form a diastereomer. The direction and extent of chemical reactions can be monitored. Rates of configurational, conformational, or tautomeric interchange in molecules can be studied, providing they fall within the NMR timescale (reciprocal of chemical shift difference in Hz between the signals of the forms).

Traceable Quantitative Analysis. The area under the NMR peaks (the integrated resonance intensity of the NMR signal) is directly proportional to the concentration of the nuclei giving the resonance. If the experiment is done properly, the proportionality constant is the same for each resonance in the spectrum. Therefore, the ratio of peaks due to different materials in a sample gives the molar ratio of the materials once the peak integrals are corrected for the number of equivalent nuclei present for each resonance. Relative concentrations can be determined directly from the relative peak areas.

$$\frac{C_t}{C_n} = \frac{\frac{I_t}{N_t}}{\frac{I_n}{N_n}} \tag{4.3}$$

Equation (4.3) describes the determination of relative concentration by QNMR where: C_t = concentration of test material; C_n = concentration of other material; I_t = integral of test material signal; N_t = relative number of nuclei responsible for test material signal; I_n = integral of other material signal; N_n = relative number of nuclei responsible for other material signal.

If each resonance in the spectrum can be accounted for and assigned to a specific molecule, then molar purity can be determined without the need for standards, assuming, of course, that all of the impurities are observable

$$\text{mol}\%\, P_t = \frac{\frac{I_t}{N_t}}{\sum \frac{I_n}{N_n}} \times 100\,. \tag{4.4}$$

Here P_t = purity of the test material; I_t = integral of test material signal; N_t = relative number of nuclei responsible for test material signal; I_n = integral of other signals; N_n = relative number of nuclei responsible for other signals.

If the molecular mass of each material is known, then the molar purities can be converted into the more useful mass-based purities by multiplying each by its molecular mass and dividing by the sum of the respective masses. If this cannot be established for a given mixture, then an internal standard procedure can be utilized to determine absolute purities where a known amount of an internal standard material is added to a solution containing a measured amount of the test material.

$$\text{mass}\%\, P_t = \frac{I_t}{I_s}\frac{N_s}{N_t}\frac{M_t}{M_s}\frac{W_s}{W_t}P_s \times 100 \tag{4.5}$$

Here: P_t = purity of the test material; I_s = integral of standard; I_t = integral of test material; N_s = relative number of nuclei responsible for standard signal; N_t = relative number of nuclei responsible for test material signal; M_s = molecular mass of standard; M_t = molecular mass of test material; W_s = mass of standard; W_t = mass of test material; P_s = purity of standard.

Several key conditions must be met for accurate QNMR determinations. Spectral signal-to-noise ratio and resolution should be optimized. To avoid effects due to relaxation of the NMR signals, the T_1 of each nucleus in the sample should be known, and either the RF pulse width or delay time between pulses should be adjusted to ensure sufficient time for full relaxation between acquisitions. Since the frequency width covered by the RF pulse is inversely related to the pulse width, care should be taken to ensure that nuclei at the extreme ends of wide spectral widths receive similar excitation intensities. The *skirts* of Lorentzian-shaped NMR signals extend to infinity, so care must be taken when comparing the integrals of peaks with very different peak widths. ^{1}H NMR spectra are complicated by signals due to coupling of the proton signals with the 1.1% of ^{13}C nuclei naturally present in organic materials. These signals must be treated consistently – either always integrated with the main signal or always omitted. Spectra acquired at higher fields help in this regard because chemical shifts (expressed in hertz) increase in proportion to the magnitude of the bulk field, whereas coupling constants (also in hertz) remain constant. Sidebands due to modulation caused by spinning the sample tube are best avoided by obtaining the spectrum in nonspinning mode with careful shimming (adjustment of the magnetic field homogeneity) to maintain resolution. For best results, only the highest grade solvents should be used in QNMR, although a spectrum of the solvent only (blank spectrum) can sometimes be used to correct for some solvent impurities. Vigilance in sample preparation is needed to avoid adventitious interferences. Sample tubes should be cleaned, then rinsed with the NMR solvent, and dried at 140 °C overnight prior to use. All transfer tools (pipettes, spatulas, and so on) must be scrupulously clean. At no time should the sample solution come into contact with the NMR tube cap or septum, as just a single contact can result in the extraction of sufficient plasticizers, surfactants and other contaminants from these materials to render a sample useless for QNMR. Similarly, touching a pipette or syringe needle with bare hands can result in the introduction of observable squalene and other components of body oil into the sample. It is common practice to make up the analytical solution in the NMR tube itself, as this minimizes inadvertent contamination. When this is done, and when accurate weighings are required (such as when using the internal standard method), care must be used to avoid weighing errors due to static charges on the tube. Commercially available radioactive sources can be used to minimize such static effects. Nevertheless, uncertainties in weighing are likely to dominate the error equation. The requirements and attributes of appropriate internal standard materials have been well-documented, but it should be apparent from (4.5) that the purity of the internal standard material and its uncertainty are key parameters. Finally, the NMR signals used for quantitation should be immune to chemical exchange. For ^{1}H NMR, this often means avoiding signals due to protons bound to oxygen and nitrogen.

With modern NMR spectrometers, optimal ^{1}H QNMR can be routinely carried out with detection and quantitation down to the 0.01% mass-fraction level with uncertainties (U_{95}) in the 0.5–1.0% range. In many cases, the detection and quantitation limits can be reduced by an order of magnitude by spending additional time on the data collection. High-field, state-of-the-art spectrometers with cryogenically cooled probes and preamplifiers can extend the useable detection and quantitation limits by another factor of four. While these limits do not begin to impinge on the ultimate sensitivities of other analytical techniques, such as mass spectrometry or fluorescence spectrometry, the use of QNMR for moderate trace-level determinations is on the increase. The coupling of NMR with chromatographic separation techniques further increases its utility.

Several review articles [4.9–34] are available.

4.1.3 Atomic Spectrometry

Atomic Absorption Spectrometry (AAS)

Principles of the Technique. The sample being analyzed is decomposed to free atoms and ions in a high-temperature flame or furnace. These atoms absorb light emitted at atomic resonance wavelengths from a hollow cathode discharge lamp with a cathode containing the analyte element. The optical absorbance is measured, and through Beer's law is proportional to concentration.

The most common atomizer is the premixed air–acetylene flame. Measurement of refractory elements requires the use of the hotter N_2O/acetylene flame. Better sensitivity is obtained using an electrically heated furnace referred to as an electrothermal atomizer or

graphite furnace. The longer residence time of atoms within the beam of hollow cathode emission yields improved sensitivity in comparison to flame atomizers.

Scope. AAS is used for quantitative elemental analysis, in other words for the determination of total concentrations of specific elements in a sample. It is commonly used for the analysis of environmental samples, industrial materials such as metal alloys, clinical or industrial hygiene measurements, and others. In general, flame AAS can be used for solution concentrations in the 10–100 mg/L range, and electrothermal AAS in the 1–100 µg/L range. AAS cannot be used for the determination of nonmetallic elements.

The instrumentation is generally less expensive than other forms of elemental analysis. In the vast majority of cases, only a single element can be measured at a time. The hollow cathode lamp must be changed to one of a different element before that element can be detected. Consequently, measurement times can be impractically long if multi-element information is required. Flame instrumentation requires training to use safely, and fumes must be exhausted.

Nature of the Sample. With few exceptions, samples are introduced into the flame or electrothermal atomizer as solutions. If the sample being analyzed is a solid, it must first be dissolved. A typical dissolution will dissolve a gram of sample in 100 mL of acidified aqueous solution. Dissolution of most samples will require hot concentrated mineral acids.

Qualitative Analysis. AAS is not typically used for qualitative analysis. The specificity (or identification) of the analyte element is provided by the atomic resonance wavelength emitted by the hollow cathode lamp.

Traceable Quantitative Analysis. AAS instrumentation must be calibrated using solutions containing known concentrations of the analyte element. Such calibration solutions are available from many commercial suppliers. These solutions are often traceable to one of a set of NIST single-element solution standard reference materials. Many AAS analyses are susceptible to matrix effects (the combined effect that the constituent species present in the sample have on the atomic absorption signal of the analyte). This will lead to measurement bias if the calibration solutions are not matched to the matrix of the sample being analyzed. The method of standard additions is an effective strategy for dealing with matrix effects. Another common source of bias is broadband molecular absorption resulting from matrix elements that are not fully atomized, or scattering of the hollow cathode beam by small particles. Most instruments utilize one of several background correction procedures to deal with such interferences. Certified reference materials are available in a wide variety of sample matrix types from NIST and other sources. These CRMs should be used to validate AAS methods.

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

Principles of the Technique. The inductively coupled plasma (ICP) used in this technique is an atmospheric-pressure argon plasma that is produced in a quartz torch connected to a radiofrequency generator. Operating RF powers are typically between 1000 and 1500 W. The aqueous liquid sample solution is injected as an aerosol into the axial channel of the ICP, where temperatures on the order of 7000 K prevail. Here the sample is decomposed to free atoms and ions, and atomic or ionic emission results as thermally populated excited states of valence electrons of the atoms or ions return to lower energy levels. A suitable optical transfer system, a monochromator or polychromator, and a detector selectively and quantitatively measure the relative emission intensities at specific analytical wavelengths. The emission intensity at an elemental emission wavelength is used as a relative measure of the concentration of that element in the sample. The method is sometimes referred to as inductively coupled plasma optical emission spectrometry (ICP OES).

Scope. ICP-AES is used for quantitative elemental analysis; in other words to determine the total concentrations of specific elements in a sample. Typical application areas include metallurgy, geological measurements, environmental monitoring, food analysis, the measurement of additives or wear metals in oil, and forensics. In general, ICP-AES is used for solution concentrations in the 1–100 mg/L range. Some nonmetallic elements (S, P) can be measured.

The instrumentation is generally more expensive than AAS, but unlike AAS it has the significant advantage of being able to detect multiple elements simultaneously. This results in significant time savings if multielement analysis is required. It is generally less expensive than ICP-MS.

Nature of the Sample. In the most common situations, samples are introduced into the ICP as solutions. If the sample being analyzed is a solid, it must first be dis-

solved. Typically, 1 g of sample is dissolved in 100 mL of acidified aqueous solution. The dissolution of samples often requires hot concentrated mineral acids.

An alternative sample introduction system uses laser ablation of solid samples to generate an aerosol of sample particles, which can be injected directly into the ICP.

Qualitative Analysis. Specific elements are identified via the known wavelengths of atomic emission lines.

Traceable Quantitative Analysis. For systems using solution sample introduction, ICP-AES instrumentation must be calibrated using solutions containing known concentrations of the analyte elements. Such calibration solutions are available from many commercial suppliers. These solutions are often traceable to one of a set of NIST single-element solution standard reference materials. The analysis of complex samples can be susceptible to matrix effects (the combined effect that the constituent species present in the sample has on the atomic emission signal of the analyte). This will lead to measurement bias if the calibration solutions are not matched to the matrix of the sample being analyzed. The method of standard additions is an effective strategy for dealing with matrix effects.

Calibrating systems using laser ablation sample introduction is more difficult. The laser ablation process is very matrix-dependent, and calibration requires the use of solid standards that match the characteristics of the sample quite closely. A measurement of the ratio of the analyte emission relative to a matrix emission line is often used in some form to improve accuracy and precision.

Spectral interference from the argon plasma emission or sample matrix components is another potential source of bias. Most systems utilize some form of spectral background correction to deal with such interferences. Certified reference materials are available in a wide variety of sample matrix types from NIST and other sources. These CRMs should be used to validate ICP-OES methods.

Spark Optical Emission Spectrometry (Spark OES)

Principles of the Technique. A high-voltage spark is generated from a cathode in an argon atomosphere to a conducting sample which acts as the anode. The sample is melted and atomized and the free atoms are excited to yield atomic emission. A suitable optical transfer system, polychromator, and detector selectively and quantitatively measure the relative emission intensity at specific analytical wavelengths. The emission intensity at an elemental emission wavelength is used as a relative measure of the concentration of that element in the sample. The method is sometimes referred to simply as optical emission spectrometry (OES) or as spark atomic emission spectrometry.

Scope. The analysis of metals is the major application area of spark OES. Tens of thousands of spark OES instruments are installed in large steel mills, aluminum smelters, and casting operations, as well as small forges and small foundries. Measurements are made for quality control, raw material testing and research. Speed of analysis, without the need for sample dissolution, is the major advantage of spark OES over ICP-OES.

Nature of the Sample. Metal samples may need to be ground flat to form a good electrode. Powders can be mixed with graphite and pressed to form a flat surface.

Qualitative Analysis. Specific elements are identified via the known wavelengths of atomic emission lines.

Traceable Quantitative Analysis. The spark sampling and excitation process is very complex and matrix-dependent. Calibration is done using large sets of Certified reference materials selected to match the type of sample to be analyzed.

As with any spectroscopic measurement, spectral interference from the sample matrix components or the spark source is a potential source of bias. Most systems utilize some form of spectral background correction to deal with such interferences.

An individual spark lasts only several microseconds, and will vaporize less than a microgram of sample. Sample inhomogeneity can impede accurate analysis of the bulk sample. Analyses are based on multiple sparks from different locations on the sample.

Glow Discharge Optical Emission Spectroscopy (GD-OES)

Principles of the Technique. A glow discharge is a reduced-pressure inert gas plasma maintained between two electrodes. Argon is typically used at absolute pressures of between a few hundred Pascals and about 2.5 kPa, and the solid sample serves as the cathode in the circuit. When the discharge is ignited, Ar^+ ions formed in the plasma are accelerated toward the sample surface by means of electric fields. At least a substantial fraction of these Ar^+ ions strike

the surface of the sample with enough kinetic energy to eject sample atoms from the surface. In this way, the solid sample is directly atomized. Once in the plasma, sputtered atoms may be electronically excited through collisions with energetic electrons and other particles. Some fraction of the excited sputtered atoms will relax to lower electronic energy levels (often the ground state) by means of photon emission. The wavelengths of these photons are characteristic of the emitting species. A grating spectrometer, with either photomultiplier tubes (PMTs) mounted on a Rowland circle, or one or more charge transfer devices (CTDs), is used to measure the intensity of the plasma emission at specific wavelengths. In this way, the elemental constituents of the solid sample can be quantitatively estimated.

A significant number of GD-OES instruments have been available commercially from several manufacturers for many years. The instruments that are currently available vary in both capabilities and costs. An instrument for a specific and routine application can be obtained for as little as $ 60 000, whereas a *fully loaded* research instrument may cost in excess of $ 200 000. There are currently more than 1000 GD-OES instruments in use around the world.

Scope. Glow discharge devices used in GD-OES may be powered by either a DC or RF power supply. In DC-GD-OES, the sample must be electrically conductive, since current must pass through it. RF-GD-OES overcomes this restriction, because in this configuration it is not necessary for net current to flow through the sample. As a result, RF-GD-OES can be applied to both electrically conductive and insulating samples.

GD-OES has traditionally been applied to bulk analysis of solids, and it is still routinely used for this purpose. More recently, GD-OES *depth profiling* (the determination of elemental composition as a function of depth) has begun to mature. In GD-OES depth profiling, registration of emission intensities over time as surface layers are *sputtered* away produces the *depth profile* of the sample. Algorithms exist to convert the resulting intensity versus time plot into elemental mass fraction versus depth. While much development remains to be done, GD-OES depth profiling is already being fairly widely applied for analyses of both electrically conductive and insulating layers on either electrically conductive or insulating substrates. Probably the widest application to date has been the analysis of galvanized coatings on steel. However, depth profiling of other sample types, such as thin films (< 10 nm thickness) and organic coatings, is being demonstrated. While bulk analysis will likely remain an important application of GD-OES, the brighter future of the technique lies in the depth profiling arena.

Some strengths of GD-OES include a relative lack of interferences and matrix effects compared to many other direct solid techniques (such as spark OES and secondary ion mass spectrometry, or SIMS). Also, limits of detection are useful for many applications (sub- to low parts per million range directly in the solid state). The ability to detect H, O and N is also an advantage. An important disadvantage is that GD-OES is inherently a *relative* technique, requiring reference materials for calibration.

Nature of the Sample. As described above, GD-OES is applicable to bulk and depth profiling analyses of electrically conductive and insulating solid samples. The RF mode is required when insulating samples are involved. Analyzed surfaces are usually flat, although special sample holders can be used for surfaces of different shapes (such as tubing).

Qualitative Analysis. Calibration of a GD-OES instrument consists of measuring emission intensities for suitable reference materials and calculating a calibration equation. Qualitative analysis can be performed without calibration, simply by noting the elemental wavelengths at which signals exist. Owing to the prominent lack of certified mass fractions for H, O and N in reference materials, GD-OES is often capable of only qualitative determinations of these nonmetallic elements.

Traceable Quantitative Analysis. Quantitative GD-OES analyses are accomplished through calibration with suitable reference materials. Accuracies improve with the level of matrix-matching of the calibrants to the unknowns. Mass fraction biases on the order of 1% relative are usually found for major and minor elements, while those observed at mass fractions below $100\,\mu g\,g^{-1}$ are normally higher (for example, $> 15\%$ relative). The accuracy of the depth axis obtained in GD-OES depth profiling may vary widely, depending upon the circumstances, but can be as good as $\pm 5\%$ relative. Whether bulk analysis or depth profiling is performed, traceability to the SI is accomplished through calibration with reference materials.

X-ray Fluorescence (XRF)

Principles of the Technique. The specimen to be analyzed can be solid (powder or bulk) or liquid (aqueous

or oil-based). It is placed in an instrument and irradiated by a primary x-ray source or a particle beam (electrons or protons). The primary radiation is absorbed and ejects electrons from their orbitals. Relaxation processes fill the holes and result in the emission of characteristic x-ray radiation. The intensity of characteristic radiation that escapes the sample is proportional to the number of atoms of each element present in the specimen. Therefore, XRF is both qualitative, using the *fingerprint* of characteristic x-rays to identify constituent elements, and quantitative, using a counting process to relate the number of x-rays detected per unit time to the total concentration of the element.

X-ray fluorescence spectrometers are available in a number of designs suited for a variety of applications and operating conditions. State-of-the-art laboratory spectrometers are typically designed as wavelength-dispersive spectrometers with a high-power tube source and a high-resolution detection system comprised of collimators or slits, a set of interchangeable crystals to diffract the characteristic x-rays according to Bragg's equation, and two or more detectors mounted on a goniometer with the crystals. Lower-cost, lower-power spectrometers consist of either smaller wavelength-dispersive spectrometers with low-power tube sources or energy dispersive spectrometers using solid-state detectors and low-power tubes or radioisotope sources. Some energy-dispersive spectrometers use beams of electrons or protons as the primary radiation source. There are even handheld units designed for field use. Given the wide variety of instruments, prices range from $ 25 000 to $ 300 000.

Scope. XRF is used for quantitative elemental analysis, typically without regard to the chemical environment of the elements in the specimen. It is a relative technique that must be calibrated using reference materials. X-rays from one element are absorbed by other elements in the specimen possibly resulting in fluorescence from those other elements. Due to these matrix effects, the best performance is obtained when the calibrant(s) are similar in overall composition to the specimen. A number of sophisticated procedures are available to compensate for matrix effects including empirical and theoretical calibration models. It is possible to obtain composition results using just theory and fundamental parameters (basic physical constants describing the interactions of x-rays with matter); however, the quality of such results varies widely. XRF measurements are also influenced by the physical nature of the specimen including particle size or grain size, mineralogy, surface morphology, susceptibility to damage by ionizing radiation, and other characteristics.

XRF is often referred as being nondestructive because it is possible to present specimens to the instrument with little or no preparation, and with little or no damage resulting from the measurement. However, x-rays cause damage at a molecular level and are not truly nondestructive, especially to organic matrices. Still, in many cases (the best example being alloys), specimens may be analyzed for other properties following XRF analysis.

XRF is at its best for rapid, precise analyses of major and minor constituents of the specimen. Spectrometers can be used for concentrations ranging from ≈ 1 mg/kg to 100% mass fraction. Analyses are accomplished in minutes and overall relative uncertainties can be limited to 1% or less. XRF is widely used for product quality control in a wide range of industries including those involving metals and alloys, mining and minerals, cement, petroleum, electronics and semiconductors.

Trace analysis is complicated by varying levels of spectral background that depend on spectrometer geometry, the excitation source, the atomic number of the analyte element, the average atomic number of the specimen, and other factors. Trace analysis below 1 mg/kg is possible using specially designed spectrometers, such as total reflection XRF, and destructive sample preparation techniques similar to other atomic emission.

Qualitative Analysis. XRF is uniquely suited for qualitative analysis with its (mostly) nondestructive nature and sensitivity to most of the periodic table (Be–U). Characteristic x-rays from each element consist of a family of lines providing unambiguous identification. Energy-dispersive spectrometers are especially well-suited for qualitative analysis because they display the entire spectrum at once. For the purpose of choosing the optimum measurement conditions, qualitative analysis is performed prior to implementation of quantitative analysis methods.

Traceable Quantitative Analysis. XRF spectrometers must be calibrated to obtain optimum accuracy. The choice of calibrants depends on the form of the specimens and the concentration range to be calibrated. Using destructive preparation techniques such as borate fusion, calibrants can be prepared from primary reference materials (elements, compounds and solutions) and the results are traceable to the SI provided the purity and stoichiometry of the reference materials are assured. The caveat is that calibrants and unknowns must

be closely matched in terms of their entire composition. The same can be accomplished for liquid materials when calibrant solutions are sufficiently similar in matrix to the unknowns.

In cases where a variety of calibrants with varying degrees of comparability to the unknowns must be used, it is necessary to apply matrix corrections. The preferred approach is to use theory and fundamental parameters to estimate the corrections. Still, some number of calibrants in the form of reference materials must be used to calibrate the spectrometer. Traceability is established through the set of reference materials to the issuing body or bodies.

Alternatives to matrix correction models are internal standards, internal reference lines and standard additions. Of course, these apply only under the appropriate circumstances in which the material to be analyzed can be prepared in some manner to incorporate the spiking material.

Several review articles [4.35–49] are available.

4.1.4 Nuclear Analytical Methods

Additional discussions of x-ray techniques are described in Sects. 4.1.4 and 4.2 [4.50–72].

Neutron Activation Analysis (NAA)

Neutron activation analysis (NAA) is an isotope-specific, multielemental, analytical method that determines the total elemental content of about 40 elements in many materials. The method is based on irradiating a sample in a field of neutrons, and measuring the radioactivity emitted by the resulting irradiation products. Typically a nuclear reactor is used as the source of neutrons, and germanium-based semiconductor detectors are used to measure the energy and intensity of the gamma radiation, which is then used to identify and quantify the analytes of interest. NAA is independent of the chemical state of the analytes, since all measurement interactions are based on nuclear and not chemical properties of the elements. In addition, both the incoming (excitation) radiation (neutrons) and the outgoing radiation (gamma rays) are highly penetrating. Due to the above characteristics, there are very few matrix effects and interferences for NAA compared to many other analytical techniques. NAA can be applied in nondestructive or *instrumental* (INAA) mode, or in a destructive mode involving dissolution and/or other chemical manipulation of the samples. The most common form of the latter mode is radiochemical NAA (RNAA), where all chemical processing is done after the irradiation step. Both INAA and RNAA are essentially free from chemical blank, since after irradiation, only the radioactive daughter products of the elements contribute to the analytical signal. In fact, for most RNAA procedures, carriers (stable forms of the elements under investigation) are added to the samples after irradiation to enhance separation and minimize losses. The amount of carrier remaining after separation can be measured to determine the chemical yield of each sample when separations are not quantitative. In other cases, a small amount of a radioactive tracer of an element under investigation can be used to determine the chemical yield.

Principles of the Technique. Most elements have one or more isotopes that will produce a radioactive daughter product upon capturing a neutron. Samples are irradiated for a known amount of time in a neutron field, removed, and then subjected to a series of gamma-ray spectrometry measurements using suitable decay intervals to emphasize or suppress radionuclides with different half-lives. Spectra of gamma-ray intensity versus energy (typically from about 70 keV–3 MeV) are collected. For RNAA measurements, virtually any type of separation procedure can be applied after irradiation. Radionuclides are identified by both their gamma-ray energy (or energies) and approximate half-lives.

Elemental content in a sample is directly proportional to the decay corrected gamma-ray count rate if irradiation and gamma-ray spectrometry conditions are held constant. The decay-corrected count rate A_0 is given

$$A_0 = \frac{\lambda C_x \, e^{\lambda t_1}}{\left(1 - e^{-\lambda \Delta}\right)\left(1 - e^{-\lambda T}\right)} , \qquad (4.6)$$

where

A_0 = decay-corrected count rate,
λ = decay constant = $\ln 2 / t_{1/2}$,
Δ = live time of count,
C_x = net counts in γ-ray peak,
t_1 = decay time to start of count,
T = irradiation time.

Scope. This method is useful for elements with isotopes that produce radioactive daughter products after neutron irradiation and decay by gamma-ray emission. Although ≈ 75 elements meet these criteria, typically 30–45 elements can be quantified instrumentally in most samples. Low-intensity signals can be lost in the continuum of background noise of the gamma-ray spec-

tra (unless radiochemical separations are employed to isolate elements of interest). Detection limits vary by approximately six orders of magnitude for INAA, and depend mainly on the nuclear properties of the elements, as well as experimental conditions such as neutron fluence rate, decay interval, and detection efficiency of the gamma-rays of interest.

Nature of the Sample. Samples of interest are encapsulated in polyethylene or quartz prior to irradiation. Typical sample sizes range from a few milligrams to about a gram, although some reactor facilities can irradiate kilogram-size samples. Because of the highly penetrating nature of both neutrons and gamma-rays, the effects of sample sizes of up to a gram are minimal unless the sample is very dense (metals) or contains large amounts of elements that are highly neutron-absorbing (B, Li, Cd, and some rare earths). Smaller sample sizes are needed for dense or highly neutron-absorbing samples. The presence of large amounts of elements that activate extremely well, such as Au, Sm, Eu, Gd, In, Sc, Mn or Co, will worsen the detection limits for other elements in the samples.

Qualitative Analysis. Radionuclides are identified by gamma-ray energies and half-lives. ^{51}Ti and ^{51}Cr have identical gamma-ray energies (320.1 keV) since they decay to the same, stable daughter product (^{51}V). However, their half-lives differ greatly: 5.76 min for ^{51}Ti and 27.7 d for ^{51}Cr. Gamma rays with an energy of 320.1 keV observed shortly after irradiation are almost entirely from Ti, while those observed even one day after irradiation are entirely from ^{51}Cr. It is possible to determine Ti in a sample with a high Cr content by first counting immediately after irradiation, counting again under the same conditions one day after irradiation, and then subtracting the ^{51}Cr contribution to the ^{51}Ti peak. In addition, many radionuclides have more than one gamma-ray, and the presence of all the intense gamma-rays can be used as confirmation.

Traceable Quantitative Analysis. Quantification for NAA can be achieved by three basic methods:

1. use of fundamental parameters;
2. by comparing to a known amount of the element under investigation, or
3. some combination of the two previous methods (the k_0 method is the best-known variant of this).

The second method, often called the comparator method, contains the most direct traceability links and will be discussed further. Typically, standards containing known amounts of the elements under investigation are irradiated and counted under the same conditions as the sample(s) of interest. Decay-corrected count rates A_0 are calculated via (4.6), and then the masses of each element in the sample(s) are calculated through (4.7). The R-values account for any experimental differences between standards and sample(s), and are normally very close to unity

$$m_{\text{unk}} = m_{\text{std}} \frac{(A_{0,\text{unk}})}{(A_{0,\text{std}})} R_\theta R_\phi R_\sigma R_\varepsilon \,, \tag{4.7}$$

where: m_{unk} = mass of an element in the unknown sample, m_{std} = mass of an element in the comparator standard, R_θ = ratio of isotopic abundances for unknown and standard, R_ϕ = ratio of neutron fluences (including fluence drop-off, self-shielding and scattering), R_σ = ratio of effective cross-sections if neutron spectrum shape differs from unk. to std., R_ε = ratio of counting efficiencies (differences due to geometry and γ-ray self-shielding).

Table 4.2 Prompt gamma activation analysis: approximate detection limits. Data from [4.61]

> 1 µg	B, Cd, Sm, Eu, Gd, Hg
1–10 µg	H, Cl, Ti, V, Co, Ag, Pt
10–100 µg	Na, Al, Si, S, K, Ca, Cr, Mn, Fe, Cu, Zn, As, Br, Sr, Mo, I, W, Au
100–1000 µg	N, Mg, P, Sn, Sb
1–10 mg	C, F, Pb, Bi

Prompt Gamma Activation Analysis (PGAA)

Principles of the Technique. The binding energy released when a neutron is captured by an atomic nucleus is generally emitted in the form of instantaneous gamma-rays. Measuring the characteristic energies of these gamma rays permits qualitative identification of the elements in the sample, and quantitative analysis is accomplished by measuring their intensity. The source of neutrons may be a research reactor, an accelerator-based neutron generator, or an isotopic source. The most sensitive and accurate analyses use reactor neutron beams with high-resolution Ge gamma-ray spectrometers. A sample is simply placed in the neutron beam, and the gamma-ray spectrum is measured during an irradiation lasting from minutes to hours. The method is nondestructive; residual radioactivity is usually negligible. Because PGAA employs nuclear (rather than chemical) reactions, the chemical form of the analyte

is unimportant. No dissolution or other sample pretreatment is required.

Scope. The sensitivity of the analysis depends on experimental conditions and on the composition of the sample matrix. For a neutron beam with a flux of $10^8\ \mathrm{cm}^{-2}\ \mathrm{s}^{-1}$ and an irradiation time of several hours, approximate detection limits are given in Table 4.2.

Nature of the Sample. Typical samples are in the range of 100–1000 mg, preferably pressed into a pellet. Samples can be smaller if the elements of interest have high sensitivities, and must be smaller if the matrix is a strong neutron absorber. Large samples may be most accurately analyzed through element ratios.

Qualitative Analysis. The energies of the peaks in the gamma-ray spectrum are characteristic of the elements. Because the spectra of most elements contain numerous peaks, elemental identification is generally positive.

Quantitative Analysis. Standards of known quantities of pure elements or simple compounds are irradiated to determine sensitivity factors. Multiple gamma rays are used for quantitation to verify the absence of interferences.

Example Spectrum. The plot shown in Fig. 4.3 is a PGAA spectrum of a fertilizer reference material. In this material, the elements H, B, C, N, P, S, Cl, K, Ca, Ti, V, Mn, Fe, Cd, Sm and Gd are quantitatively measurable.

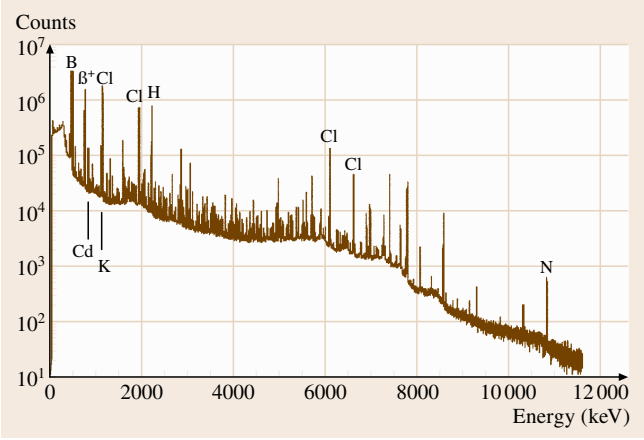

Fig. 4.3 Prompt gamma activation analysis

Neutron Depth Profiling

Neutron depth profiling (NDP) is a method of near-surface analysis for isotopes that undergo neutron-induced positive Q-value (exothermic) charged particle reactions, for example (n,α), (n,p). NDP combines nuclear physics with atomic physics to provide information about near-surface concentrations of certain light elements. The technique was originally applied in 1972 by *Ziegler* et al. [4.62] and independently by *Biersack* and *Fink* [4.63]. *Fink* [4.64] has produced an excellent report giving many explicit details of the method. The method is based on measuring the energy loss of the charged particles as they exit the specimen. Depending on the material under study, depths of up to 10 µm can be profiled, and depth resolutions of the order of 10 nm can be obtained. The most studied analytes have been boron, lithium, and nitrogen in a variety of matrices, but several other analytes can also be measured. Because the incoming energy of the neutron is negligible and the interaction rate is small, NDP is considered a non-destructive technique. This allows the same volume of sample to receive further treatment for repeated analysis, or to be subsequently analyzed using a different technique that might alter or destroy the sample.

Principles of the Technique. Lithium, boron, nitrogen, and a number of other elements have an isotope that undergoes an exoergic charged particle reaction upon capturing a neutron. The charged particles are protons or alpha particles and an associated recoil nucleus. The energies of the particles are determined by the conservation of mass-energy and are predetermined for each reaction (for thermal neutrons, the added energy brought in by the neutron is negligible). As the charged particle exits the material, its interaction with the ma-

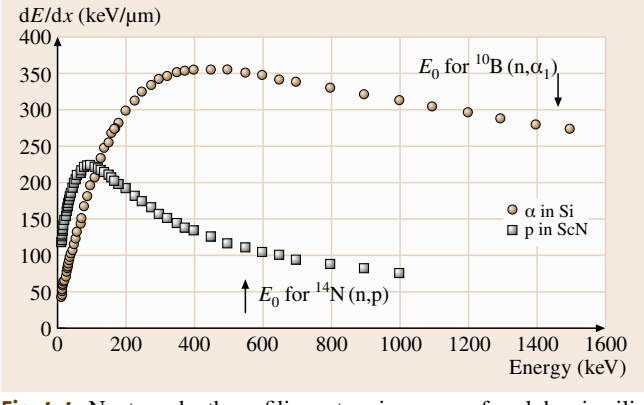

Fig. 4.4 Neutron depth profiling: stopping power for alphas in silicon and protons in ScN

trix causes it to lose energy, and this energy loss can be measured and used to determine the depth of the originating reaction. Because only a few neutrons undergo interactions as they penetrate the sample, the neutron fluence rate is essentially the same at all depths. The depth corresponding to the measured energy loss is determined by using the characteristic stopping power of the material. The chemical or electrical state of the target atoms has an inconsequential effect on the measured profile in the NDP technique. Only the concentration of the major elements in the material is needed to establish the depth scale through the relationship to stopping power.

Mathematically, the relationship between the depth and residual energy can be expressed as

$$x = \int_{E(x)}^{E_0} \frac{\mathrm{d}E}{S(E)} \,, \tag{4.8}$$

where x is the path length traveled by the particle through the matrix, E_0 is the initial energy of the particle, $E(x)$ is the energy of the detected particle, and $S(E)$ is the stopping power of the matrix. Examples of the relationship between x and $E(x)$ are displayed in Fig. 4.4 for ^{10}B(n,α) in silicon and ^{14}N(n,p) in ScN. For the boron reaction, ^{10}B(n,α)^{7}Li, there are two outgoing alpha particles with energies of 1.472 MeV (93% branch) and 1.776 MeV (7%), and two corresponding recoil ^{7}Li nuclei with energies of 0.840 and 1.014 MeV. For the nitrogen reaction, ^{14}N(n,p)^{14}C, there is a 584 keV proton and a 42 keV ^{14}C recoil. A silicon surface barrier detector detects particles escaping from the surface of the sample. The charge deposited in the detector is directly proportional to the energy of the incoming particle.

Scope. A principal limitation of the technique is that it can only be applied to a few light elements. The most commonly analyzed are boron, lithium and nitrogen. However, as a result, very few interfering reactions are encountered. Furthermore, since the critical parameters in the technique are nuclear in origin, there is no dependence upon the chemical or optical characteristics of the sample. Consequently, measurements are possible at the outer few atomic layers of a sample or through a rapidly changing composition such as at the interface between insulating and conducting layers, and across chemically distinct interfaces. In contrast, measurement artifacts occur with surface techniques such as secondary ion mass spectrometry and Auger electron spectrometry when the sample surface becomes charged and the ion yields vary unpredictably.

Nature of the Sample. Samples of interest are usually thin films, multilayers or exposed surfaces. Because of the short range of the charged particles, only depths to about 10 μm can be analyzed. The samples are placed in a thermal neutron beam and the dimensions of the beam determine the maximum area of the sample that can be analyzed in a single measurement. Some facilities have the ability to scan large-area samples. The analyzed surface must be flat and smooth to avoid ambiguities caused by surface roughness. All samples are analyzed in vacuum, so the samples must be nonvolatile and robust enough to survive the evacuation process. Some samples may become activated and require some decay time before being available for further experimentation. The latter condition is the only barrier to the entire process being completely nondestructive.

Qualitative Analysis. Most of the interest in the technique relates to determining the shape of the distribution of the analyte and how it responds to changes in its environment (annealing, voltage gradients and so on). Determining the shape of the distribution involves determining the energy of the particles escaping from the surface and comparing with the full energy of the reaction. The detector can be calibrated for the full energy by measuring a very thin surface deposit of the analyte in question. The detector is typically a surface-barrier detector or another high-resolution charged particle detector. The difference between the initial energy of the particle and its measured energy is equal to the energy loss, and with (4.8) it yields the depth of origin. The depth resolution varies from a few nanometers to a few hundred nanometers. Under optimum conditions the depth resolution for boron in silicon is approximately 8 nm.

Stopping powers for individual elements are given in compilations like that of *Ziegler* [4.65]. Because the analytic results are obtained in units of areal density (atoms per square centimeter), a linear depth scale can be assigned only if the volume density of the material remains constant and is known. Consequently, the conversion of an energy loss scale to a linear depth axis is only as accurate as the knowledge of the volume density. By supplying a few physical parameters, customized computer programs are used to convert the charged particle spectrum to a depth profile in units of concentration and depth. A Monte Carlo program, SRIM [4.66], can also be used to provide stopping

power and range information. Even if the density is not well-known, mass fraction concentration profiles can be accurately determined even through layered materials of different density. In many cases, it is the mass fraction composition that is the information desired from the analysis.

Traceable Quantitative Analysis. To compare concentration profiles among samples, both the charged particle spectrum and the neutron fluence that passes through each sample are monitored and recorded. The area analyzed on a sample is defined by placing an aperture securely against the sample surface. This aperture need only be thick enough to prevent the charged particle from reaching the detector and can therefore be very thin. Neutron collimation is used to reduce unwanted background, but it does not need to be precise. The absolute area defined by the aperture need not be accurately known as long as the counting geometry is constant between samples. The neutron fluence recorded with each analysis is used to normalize data from different samples. In practice, a run-to-run monitor that has a response proportional to the total neutron fluence rate is sufficient to normalize data taken at differing neutron intensities and time intervals. To obtain a traceable quantitative analysis of the sample, a spectrum should be obtained using a sample of known isotopic concentration, such as the NIST SRM 2137 boron implanted in silicon standard for calibrating concentration in a depth profile. When determining an NDP profile it should be remembered that only the isotopic concentration is actually determined and that the elemental profile is inferred.

Photon Activation Analysis (PAA)

Principles of the Technique. PAA is a variant of activation analysis where photons are used as activating particles. The nuclear reactions depend on the atomic number of the target and on the energy of the photons used for irradiation. The source of photons for PAA is nearly always the bremsstrahlung radiation produced with electron accelerators. The photon energies are commonly 15–20 MeV, predominantly inducing the (γ,n) reaction. Other reactions that can be used include (γ,p), (γ,2n), and (γ,α). PAA is very similar to neutron activation analysis (NAA) in that the photons can completely penetrate most samples. Thus procedures and calculations are similar to those used in NAA. The method has constraints due to the stability and homogeneity of the photon beam, with inherent limitations to the comparator method. Recent developments in bremsstrahlung target technology have achieved improvements in the photon source that greatly benefit the precision and accuracy of the method [4.59]. A detailed discussion of PAA has been given by *Segebade* et al. [4.67].

Scope. The method is complementary to INAA, and the determination of light elements C, N, O and F are good examples of PAA where detection limits of $< 0.5\,\mu g$ are possible. A few heavy metal elements can be determined in biological and environmental materials with similar sensitivity, such as Ni, As, and Pb; the latter cannot be determined by thermal NAA. One reaction with lower energy photons is the $^{9}Be(\gamma,n)^{10}Be \rightarrow 2\ ^{4}He +$ 2n reaction because of the low neutron binding energy of the Be. The reaction can be induced by the 2.1 MeV gamma rays from ^{124}Sb and measured through the detection of the neutrons. (The same reaction is also used as a neutron source).

Nature of the Sample. Samples are commonly in solid form, requiring little or no preparation for analysis. Metals, industrial materials, environmental materials and biological samples can be characterized in their original form.

Qualitative Analysis. The (γ,n) reaction leaves the product nucleus proton-rich, consequently the analytical nuclide is frequently a positron emitter. This requires discrimination by half-life or radiochemical separation for element-specific characterization. Heavier elements can form product nuclides which emit their own characteristic gamma rays, rather than just positron annihilation radiation.

Traceable Quantitative Analysis. The comparator method of activation analysis relates directly the measured gamma rays of a sample to the measured gamma rays of a standard with a known element content (4.7). Spectral interferences, fluence differences in sample and standard, and potential isotopic differences must be carefully considered.

Charged Particle (Beam) Techniques

Principles of the Technique. In charged particle activation analysis (CPAA), the activating particles, such as protons, deuterons, tritons, ^{3}He, α- and higher atomic number charged particles are generated by accelerators. The type of nuclear reaction induced in the sample nuclei depends on the identity and energy of the incoming charged particle. Protons are selected in many instances

because they can be easily accelerated and have low Coulomb barriers. The (p,n) and (p,γ) reactions result most often with protons up to about 10 MeV in energy. Higher energy protons may also induce (p,α), (p,d) or (p,2n) reactions. Larger incident particles require higher energies to overcome the Coulomb barrier. They then deposit higher energies in the target nucleus during reaction, which leads to a greater variety of pathways for the de-excitation of the activated nucleus. Therefore, a great variety of reactions and measurement options are available to the analyst in CPAA. CPAA has been discussed in more detail by *Strijckmans* [4.68].

Particle-induced x-ray emission (PIXE) is a combined process, in which continuum and characteristic x-rays are generated through the recombination of electrons and electron vacancies produced in ion–atom collision events when a beam of charged particles is slowed down in an object. Usually protons of energies between 0.1–3 MeV are utilized; the energies typically depend on the accelerator type and are selected to minimize nuclear reactions. The target elements emit characteristic x-ray lines corresponding to the atomic number of the element. A detailed introduction to the technique and its interdisciplinary applications is given by *Johansson* et al. [4.69].

Scope. CPAA can be regarded as a good complement to neutron activation analysis (NAA), since elements that are measured well are quite different from those ordinarily determined by NAA. The low Coulomb barriers and low neutron capture cross-sections of light elements make CPAA a good choice for the nuclear analysis of B, C, N, O, and so on. PIXE is generally applicable to elements with atomic numbers $11 \leq Z \leq 92$. Because charged particles may not penetrate the entire sample as neutrons do, CPAA or PIXE is often used for determinations in thin samples or as a surface technique. The ability to focus charged particle beams is widely used in applications for spatial analysis. Elaborate ion-optical systems of particle accelerators composed of focusing and transversal beam scanning elements offer an analytical tool for lateral two-dimensional mapping of elements in micro-PIXE.

Nature of the Sample. Samples are commonly in solid form, requiring no or little preparation for analysis. Metals, industrial materials, environmental materials and biological samples can be characterized in their original solid form. However, many facilities require that the sample is irradiated in a vacuum chamber connected to the accelerator beam line. Samples with volatile constituents and liquid samples require that the beam is extracted from the beam line through a thin window. Activation and x-ray production in the window and surrounding air significantly increases the background in prompt gamma ray and x-ray spectra. An additional restriction may be imposed on sample materials by the sensitivity of the sample to local heating in the particle beam.

Qualitative Analysis. The selection of nuclear reaction parameters in CPAA permits the formation of rather specific product nuclides which emit their own characteristic gamma rays for unique identification. PIXE offers direct identification of elements via their characteristic K and L series x-rays.

Traceable Quantitative Analysis. For CPAA, the interaction of the charged particle with the sample requires a modification of the activation equation, introduced in Sect. 4.2.4. The particles passing through a sample lose energy, so the cross-section for the nuclear reaction changes with depth. The expression for the produced activity in NAA has to be modified to account for this effect

$$A(t) = nI \int_0^R \sigma(x)\,\mathrm{d}x \,. \tag{4.9}$$

Here I is the beam intensity (replacing the neutron flux Φ), n is the density of target nuclides, and R is the penetration range determined by the stopping power of the medium according to Sect. 4.2.4.

As with NAA, the fundamental equation is not used directly in practice, but a relative standardization (comparator) method is used. A working equation for the comparator method is

$$m_x = m_\mathrm{s} \frac{A_x I_\mathrm{s} R_\mathrm{s}}{A_\mathrm{s} I_x R_x} \,. \tag{4.10}$$

Here we have omitted the saturation, decay, and counting factors, which should be applied the same as in the case of NAA (4.7).

Like NAA, PIXE can be described by sets of equations relating to all of the physical parameters applicable in the excitation process. However, accurate calibration of a PIXE system is extremely difficult. The comparator method suffers from the fact that only one sample, either the unknown or the standard, can be irradiated at a given time. Thin target yields for thin homogeneous samples with negligible energy loss of the bombarding particle and no absorption of x-rays in the sample can be calculated and normalized

for standards and unknowns. For a thick homogeneous sample, thick target yields can be calculated if the composition is known. The comparator method is further affected by the problem of insufficient matrix match between unknown and standard sample here. Often internal standards, such as homogeneously mixed in yttrium, provide an experimental calibration method with a potential limit to uncertainties of 3–5%.

Activation Analysis with Accelerator-Produced Neutrons

Principles of the Technique. This nuclear analytical method is based on small, low-voltage (≈ 105–200 kV) accelerators producing 3 and 14 MeV neutrons via the $^{2}H(d,n)^{3}He$ and $^{3}H(d,n)^{4}He$ reactions, respectively. Principles of operation and output characteristics of these neutron generators have been described in the past [4.70] and were recently updated in a technical document [4.55]. The NAA procedures follow the same principles as those with thermal neutrons. The high-energy neutrons can be used to interact directly with target nuclides in fast neutron activation analysis (FNAA), or they are moderated to thermal energies before interacting with a sample like in conventional NAA. Hence, the principal neutron energies of interest obtained from neutron generators are ≈ 14 MeV, ≈ 2.8 MeV, and ≈ 0.025 eV (thermal). The different nuclear reactions of the generator neutrons are listed here in the approximate order of increasing threshold energy: (n,γ), (n,n′,γ), (n,p), (n,α), and (n,2n). The (n,γ) reaction is exoergic and the cross-section in most cases decreases with increasing neutron energy. Nevertheless, some nuclides have high resonance absorption at certain neutron energies and FNAA is used in their determination. The (n,n′,γ) reactions are slightly endoergic; most can be induced by the 3 MeV neutrons. Only a limited number of nuclides, however, have longer lived isomeric states that can be measured after irradiation. The (n,p), (n,α), and (n,2n) reactions are predominantly endoergic and generally occur with the 14 MeV neutrons only. With this wide array of selectable parameters, highly specialized applications have been developed.

Scope. The FNAA methods based on small neutron generators play an important role in the development of new technologies in process and quality control systems, exploration of natural resources, detection of illicit traffic materials, transmutation of nuclear waste, fusion reactor neutronics, and radiation effects on biological and industrial materials. A considerable number of systems have been specifically tailored to the important application of oxygen determination via the $^{16}O(n,p)^{16}N$ ($T_{1/2} = 7.2$ s) reaction. This outstanding analytical application for the direct, nondestructive determination of oxygen is discussed here in more detail. The procedure has been documented in an evaluated standard test method (ASTM).

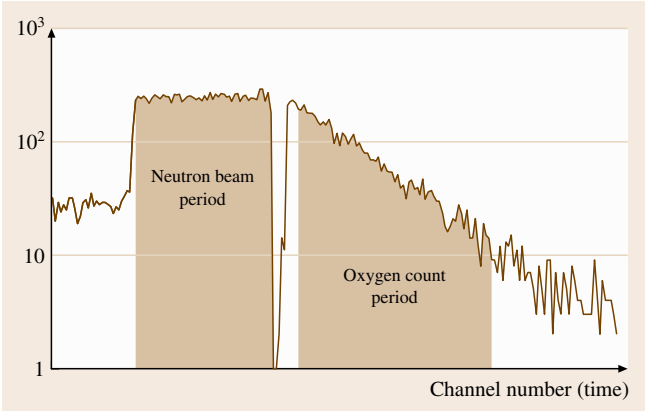

Fig. 4.5 Activation analysis with accelerator-produced neutrons: multiscaling spectrum of neutron beam monitor and oxygen gamma-ray counts in the FNAA of coal

Nature of the Sample. Samples are in solid or liquid form, requiring little or no preparation for analysis. Metals, industrial materials, environmental materials and biological and other organic samples can be characterized in their original forms. For highly sensitive oxygen determinations, samples are commonly prepared under inert gas protection and sealed in low-level oxygen irradiation containers. Comparator standards are commonly prepared from stoichiometric oxygen-containing chemicals measured directly or diluted with relatively oxygen-free filler materials.

Qualitative Analysis. In general applications of FNAA, the activation products are identified by their unique nuclear decay characteristics. The oxygen analysis commonly utilizes the summation of all gamma rays above ≈ 4.7 MeV; the specificity of the accumulated counts is ascertained by decay curve analysis.

Traceable Quantitative Analysis. FNAA follows the general principles of NAA for quantitative analysis. The specific nature of the neutron flux distributions and intensities during an activation cycle with a generator and the produced nuclides, which are frequently short-lived,

however, require specific measures to control sources of uncertainty.

The 14 MeV FNAA method has been used to determine the uptake of oxygen in SRM 1632c trace elements in coal (bituminous) [4.71]. In this application it was expected to quantify a relative change of 5% in the oxygen content (≈ 12% mass fraction) in the SRM. Automated irradiation and counting cycles alternate samples and standards and achieve high precision though multiple (ten) passes for each sample and standard. Figure 4.5 illustrates the signals recorded in the analyzer for each pass. The duration of the irradiation and its intensity is recorded from a neutron monitor, while the oxygen gamma rays are obtained from a matched pair of NaI(Tl) photon detectors after travel of the sample from the irradiation to the counting position. An on-line laboratory computer controls the process and quantitatively evaluates the data [4.72].

4.1.5 Chromatographic Methods

Gas Chromatography (GC)

Principles of the Technique. Gas chromatography (GC) can be used to separate volatile organic compounds. A gas chromatograph consists of a flowing mobile phase (typically helium or hydrogen), an injection port, a separation column containing the stationary phase, and a detector. The analytes of interest are partitioned between the mobile (inert gas) phase, and the stationary phase. In capillary gas chromatography, the stationary phase is coated on the inner walls of an open tubular column typically comprised of fused silica. The available stationary phases include methylpolysiloxanes with varying substituents, polyethylene glycols with different modifications, chiral columns for separations, as well as other specialized stationary phases. The polarity of the stationary phase can be varied to effect the separation.

The suite of gas chromatographic detectors includes the flame ionization detector (FID), the thermal conductivity detector (TCD or hot wire detector), the electron capture detector (ECD), the photoionization detector (PID), the flame photometric detector (FPD), the atomic emission detector (AED), and the mass spectrometer (MS). Except for the AED and MS, these detectors produce an electrical signal that varies with the amount of analyte exiting the chromatographic column. In addition to producing the electrical signal, the AED yields an emission spectrum of selected elements in the analytes. The MS, unlike other GC detectors, responds to mass, a physical property common to all organic compounds.

Scope. Capillary chromatography has been used for the separation of complex mixtures, components that are closely related chemically and physically, and mixtures that consist of a wide variety of compounds. Because the separation is based on partitioning between a gas phase and stationary phase, the analytes of interest must volatilize at temperatures obtainable by GC injection port temperatures (typically 50–300 °C) and be stable in the gas phase.

Samples may be introduced using split, splitless or on-column injectors. During a split injection, a portion of the carrier gas is constantly released through a splitter vent located at the base of the injection port, so that the same proportion of the sample injected will be carried out of the splitter vent upon injection. For applications where sensitivity or degradation in the injection port is not an issue, split injections are performed. During a splitless injection, the splitter vent is closed for a specified period of time following injection and then opened. For applications where sensitivity is an issue but degradation in the injection port is not an issue, and there are a lot of coextractables in the sample, splitless injection is used. Inlet liners are used for both split and splitless injections. For on-column injection, the column butts into the injection port so that the syringe needle used for injections goes into the head of the column. In this case, all of the sample is deposited onto the head of the column typically at an injection temperature below the boiling point of the solvent being used. For applications where sensitivity and degradation in the injection port are both issues and there is a limited amount of coextractables in the sample, on-column injection is used.

Nature of the Sample. The samples are introduced into the gas chromtograph as either gas or liquid solutions. If the sample being analyzed is a solid, it must first be dissolved into a suitable solvent, or the analytes of interest in the matrix must be extracted into a suitable solvent. In the case of complex matrices, the analytes of interest may be isolated from some of the coextracted material using various steps, including but not limited to size exclusion chromatography, liquid chromatography and solid-phase extraction.

Qualitative Analysis. Gas chromatography provides several types of qualitative information simultaneously. The appearance of the chromatogram is an indication of the complexity of the sample. The retention times of the analytes allow classification of various components roughly according to volatility. The rate at which

a component travels through the GC system (retention time) depends on factors in addition to volatility, however. These include the polarity of the compounds, the polarity of the stationary phase, the column temperature, and the flow rate of the gas (mobile phase) through the column. GC-AED and GC/MS provide additional qualitative information.

Traceable Quantitative Analysis. Regardless of the detector being used, GC instrumentation must be calibrated using solutions containing known concentrations of the analyte of interest along with internal standards (surrogates) that have been added at a known concentration. The internal standards (surrogates) chosen should be chemically similar to the analytes of interest, and for many of the GC/MS applications are isotopically labeled analogs of one or more of the analytes of interest. Approximately the same quantity of the internal standard should be added to all calibration solutions and *unknown* samples within an analysis set. Calibration may be performed by constructing a calibration curve encompassing the measurement range of the samples or by calculating a response factor from measurements of calibration solutions that are very similar in concentration to or closely bracket the sample concentration for the analyte of interest. For more detail on quantitative analysis as it relates to chromatography, see the section in this chapter on liquid chromatography.

Certified reference materials are available in a wide variety of sample matrix types from NIST and other sources. These CRMs should be used to validate the entire GC method, including extraction, analyte isolation and quantification.

Liquid Chromatography (LC)

Liquid chromatography (LC) is a method for separating and detecting organic and inorganic compounds in solution. The technique is broadly applicable to polar, nonpolar, aromatic, aliphatic and ionic compounds with few restrictions. Instrumentation typically consists of a solvent delivery device (a pump), a sample introduction device (an injector or autosampler), a chromatographic column, and a detector. The flexibility of the technique results from the availability of chromatographic columns suited to specific separation problems, and detectors with sensitive and selective responses. The goal of any liquid chromatographic method is the separation of compounds of interest from interferences, in either the chromatographic and/or detection domains, in order to achieve an instrumental response proportional to the analyte level.

Principles of the Technique. Retention in liquid chromatography is a consequence of different associations of solute molecules in dissimilar phases. In the simplest sense, all chromatographic systems consist of two phases: a fixed *stationary phase* and a moving *mobile phase*. The diffusion of solute molecules between these phases usually occurs on a time scale much more rapid than that associated with fluid flow of the mobile phase. Differential association of solute molecules with the stationary phase retards these species to different extents, resulting in separation. Retention processes depend on a complex set of interactions between solute molecules, stationary phase ligands and mobile phase molecules; the characteristics of the column (such as the physical and chemical properties of the substrate, the surface modification procedures used to prepare the stationary phase, the polarity, and so on) also provide a major influence on retention behavior. Two modes of operation can be distinguished: reversed-phase liquid chromatography (RPLC) and normal-phase LC. For normal-phase LC, the mobile phase is less polar than the stationary phase; the opposite situation exists with RPLC. Column choice is critical when developing an LC method. Most separations are performed in the reversed-phase mode with C_{18} (octadecylsilane, ODS) columns. An instrumental response proportional to the analyte level typically results from spectrometric detection, although other forms of detection exist. Common detectors include UV/Vis absorbance, fluorescence (FL), electrochemical (EC), refractive index (RI), evaporative light scattering (ELSD), and mass spectrometric (MS) detection.

Scope. Liquid chromatography is applicable to compounds that are soluble (or can be made soluble by derivatization) in a suitable solvent and can be eluted from a chromatographic column. Accurate quantification requires the resolution of constituents of interest from interferences. Liquid chromatography is often considered a low-resolution technique, since only about 50–100 compounds can be separated in a single analysis; however, selective detection can be implemented to improve the overall resolution of the system. Recent emphasis is on the use of mass spectrometry for selective LC detection. In general, liquid chromatographic techniques are most suited to thermally labile or nonvolatile solutes that are incompatible with gas phase separation techniques (such as gas chromatography).

Nature of the Sample. Liquid chromatography is relevant to a wide range of sample types, but in all cases

samples must be extracted or dissolved in solution to permit introduction into the liquid chromatograph. To reduce sample complexity, enrichment (clean-up) of the samples is sometimes carried out by liquid–liquid extraction, solid-phase extraction, or LC fractionation. Sample extracts should be miscible with the mobile phase, and typically small injection volumes (1–20 μL) are employed. Solvent exchange can be carried out when sample extracts are incompatible with the mobile phase composition.

Qualitative Analysis. Liquid chromatography is sometimes used for tentative identification of sample composition through comparison of retention times with authentic standards. Identifications must be verified by complementary techniques; however, disagreement in retention times is usually sufficient to prove the absence of a suspected compound.

Traceable Quantitative Analysis. Liquid chromatography is a relative technique that requires calibration. The processes of calibration and quantification are similar to those used in other instrumental techniques for organic analysis (such as gas chromatography, mass spectrometry, capillary electrophoresis, and related hyphenated techniques). The quantitative determination of organic compounds is usually based on the comparison of instrumental responses for unknowns with calibrants. Calibrants are prepared (usually on a mass fraction basis) using reference standards of known (high) purity. This comparison is made by using any of several mathematical models. Linear relationships between response and analyte level are often assumed; however, this is not a requirement for quantification, and nonlinear models may also be used.

Several approaches to quantification are potentially applicable: the external standard approach, the internal standard approach, and the standard addition approach. The external standard approach is based on a comparison of absolute responses for analytes in the calibrants and unknowns. The internal standard approach is based on a comparison of relative responses of the analytes to the responses of one or more compounds (the internal standard(s)) added to each of the samples and calibrants. The standard addition approach is based on one or more additions of a calibrant to the sample, and may also utilize an internal standard.

The external standard approach is often used when an internal standard is not available or cannot be used, or when the masses of standard and unknown samples can easily be controlled or accounted for. The external standard approach demands care since losses from sample handling or sample introduction will directly influence the final results. All volumes (or masses) must be accurately known, and sample transfers must be quantitative.

The internal standard approach utilizes one or more constituents (the internal standard(s), not present in the unknown samples) which are added to both calibrants and unknowns. Calculations are based on relative responses of the analytes to these internal standards. The use of internal standards lessens the need for quantitative transfers and reduces biases from sample processing losses. Internal standards should (ideally) have properties similar to the analytes of interest; however, even internal standards with unrelated properties may provide benefits as *volume correctors*. An isotopic form of the analyte of interest is used for isotope dilution methods. A mass difference of at least 2 and substitution at nonlabile atoms is typically required for mass spectrometric methods. Separation of isotopically labeled species (required for non-mass selective detection) is sometimes possible for deuterated species when the number of deuterium atoms is 8–10 or greater. Separation of the internal standard is required when detection is nonselective, as with ultraviolet absorbance detection. For techniques that utilize selective detection (such as mass spectrometry), separation of the internal standard is not required, and often it is desired that the internal standard and analyte coelute for improved precision (as in isotope dilution approaches).

The standard addition approach is based on the addition of a known quantity(s) of a calibrant to the unknown (with or without addition of an internal standard). At least two sample levels must be prepared for each unknown; one sample can be the unspiked unknown. Since separate calibrations are carried out for each unknown sample, this approach is labor-intensive.

Internal and external standard approaches to quantification can utilize averaged response factors, a zero intercept linear regression model, a calculated intercept linear regression model, or another nonlinear model. The responses can be unweighted or weighted. The model utilized should be evaluated as appropriate for the measurement problem.

The number and level of calibrants used depends on the measurement problem. When the level(s) of the unknown can be estimated, calibrants should be prepared to approximate this level(s). Preparation of calibrants in this way minimizes the issue of response linearity. When less is known about the unknowns, or when unknowns are expected to span a concentration range, calibrants should be prepared to span this range. It is un-

desirable to extrapolate to concentrations outside of the calibration interval. When possible, prepare calibrants by independent gravimetric processes and avoid serial dilutions or use of stock solutions.

When internal standards are used as part of the method, levels should be selected to approximate the levels of components being measured. The response for the internal standard should be the same or similar for calibrants and unknowns, and the ratio of internal standard and analyte(s) should be similar. When possible, the absolute response for analytes and internal standards should be significantly greater than the noise level. If the analyte level(s) are low in the unknown samples, the internal standard should be added at higher levels (measurement precision should be enhanced if the internal standard is significantly greater than the noise level).

Capillary Electrophoresis

Principles of the Technique. Capillary electrophoresis (CE) refers to a family of techniques that are based upon the movement of charged species in the presence of an electric field. A simplified diagram of a CE instrument is shown in Fig. 4.6. When a voltage is applied to the system, positively charged species move toward the negatively charged electrode (cathode), while negatively charged species migrate toward the positively charged electrode (anode). Neutral species are not attracted to either electrode.

Separations are typically performed in fused silica capillaries with an internal diameter of 25–100 μm and a length of 25–100 cm. The capillary is filled with a buffer, and the applied voltage generally ranges from 10–30 kV. A number of different detection strategies

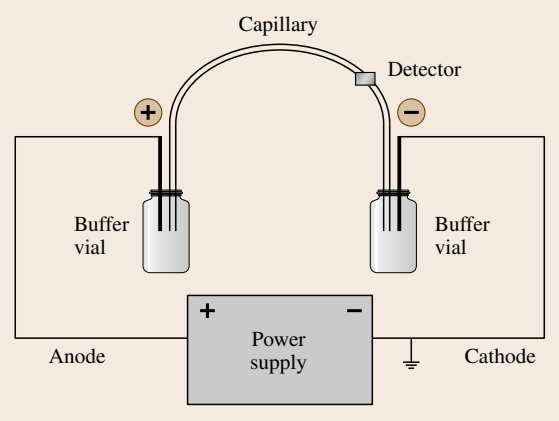

Fig. 4.6 Capillary electrophoresis: diagram of CE instrumentation

are available, including UV absorbance, laser-induced fluorescence, and mass spectrometry.

Scope. CE is applicable to a wide range of pharmaceutical, bioanalytical, environmental and forensic analyses. Various modes of CE are employed depending upon the type of analyte and the mechanism of separation. Capillary zone electrophoresis (CZE) is the most widely used mode of CE and relies upon differences in size and charge between analytes at a given pH to achieve separations. Some of the demonstrated applications of CZE include the analysis of drugs and their metabolites, peptide mapping, and the determination of vitamins in nutritional supplements. Neutral compounds are typically resolved using micelles, through a technique known as micellar electrokinetic capillary chromatography (MECC or MEKC). Both CZE and MEKC have also been utilized for enantioselective separations. Capillary gel electrophoresis (CGE) has been used extensively for the separation of proteins and nucleic acids. The gel network acts as a sieve to separate components based on size. Capillary isoelectric focusing (CIEF) separates analytes on the basis of their isoelectric points and incorporates a pH gradient. This technique is commonly used for the separation of proteins. Capillary isotachophoresis (CITP) utilizes a combination of two buffer systems and is sometimes used as a preconcentration method for other CE techniques.

CE has been viewed as an alternative to liquid chromatography (LC), although CE is not yet as well-established as LC. CE typically provides a higher efficiency than LC and has lower sample consumption. In addition, the various modes of CE offer flexibility in method development. Because CE utilizes a different separation mechanism to LC, it can be viewed as an orthogonal technique that provides complementary information to LC analyses. Currently, the primary limitations of CE involve sensitivity and reproducibility issues, but improvements continue to be made in these areas.

Nature of the Sample. Samples for CE cover a wide range and include matrices such as biological fluids, protein digests and pharmaceutical compounds. Depending on the sample matrix, the sample may be injected directly or may be diluted in water or the run buffer. Certain sample preparation techniques can be utilized to optimize sensitivity. Derivatization of the analytes is often required for laser-induced fluorescence detection.

Qualitative Analysis. CE is particularly applicable to qualitative analyses of peptides and proteins. The high efficiency of CE yields separations of even closely related species, and the *fingerprints* resulting from the analysis of two different samples can be compared to reveal subtle differences.

Traceable Quantitative Analysis. Quantification in CE is generally performed by preparing calibration solutions of the analyte(s) of interest at known concentrations and comparing the peak areas obtained for known and unknown solutions. Quantification approaches used in CE are generally similar to those used in LC. One unique aspect of CE is the fact that the peak area is related to the migration velocity of the solute. Corrected peak areas, obtained by dividing the peak area by the migration time of the analyte, improve quantitative accuracy in CE.

Liquid Chromatography/Mass Spectrometry (LC/MS) and LC/MS/MS

The combination of liquid chromatography (LC) with mass spectrometry (MS) is a powerful tool for the determination of organic and organometallic species in complex matrices. While LC is sometimes combined with ICP-MS for elemental analysis, this section will focus on combining LC with MS using either electrospray ionization (ESI)[1] or atmospheric pressure chemical ionization (APCI), the most widely used approaches for the determination of organic species. Generally, reversed-phase LC using volatile solvents and additives is combined with a mass spectrometer equipped with either an ESI or an APCI source. ESI is the favored approach for ionic and polar species, while APCI may be preferred for less polar species. If the mass spectrometer has the ability to perform tandem mass spectrometry (MS/MS) using collision-induced dissociation, analysis of daughter ions adds additional specificity to the process.

Principles of the Technique. The principles of liquid chromatography are covered in the liquid chromatography section. For LC/MS, the effluent from the LC column flows into the source of the mass spectrometer. For ESI the effluent is sprayed out of a highly charged orifice, creating charged clusters that lose solvent molecules as they move from the orifice, resulting in charged analyte molecules. For APCI, a corona discharge is used to ionize solvent molecules that act as chemical ionization reagents to pass charges to the analyte molecules. Desolvated ions pass into the MS vacuum system through a pinhole. The ions may be separated by various devices including quadrupole, ion trap, magnetic sector, time-of-flight, or ion cyclotron resonance devices. The principles of operation of these devices are beyond the scope of this discussion. Ions are generally detected using electron or ion multipliers.

Scope. LC/MS and LC/MS/MS have been used to determine organic species ranging from highly polar peptides and oligonucleotides to low-polarity species such as nitrated polycyclic aromatic hydrocarbons in virtually any matrix imaginable. For many polar substances such as drug metabolites or hormones, LC/MS has largely supplanted GC/MS as the approach of choice for two reasons. Sample preparation is much simpler with LC/MS in that the analyte is generally not derivatized and analyte isolation from the matrix is often faster. Nevertheless, with most complex matrices, some sample processing is generally necessary before the sample is introduced into the LC/MS. Secondly, sensitivity is often greater, resulting in better quantification of very low concentrations. Because both ESI and APCI are soft ionization techniques, there is little fragmentation observed in contrast to what is seen for many analytes in GC/MS. This can be either advantageous or a negative feature. The ion intensity is concentrated in far fewer ions, thus improving sensitivity. However, if there are interferences at the ions being monitored, there are not usually any alternatives ions that can be used for measurement, such as there often are with GC/MS. However, if MS/MS is available, there is usually a parent–daughter combination that is free from significant interference. For both LC/MS and LC/MS/MS, use of an isotope-labeled form of the analyte as the internal standard is the preferred approach for quantification. However, satisfactory results are sometimes possible with a close analog of the analyte, provided that they can be separated.

Qualitative Analysis. LC/MS can be a useful tool for qualitative analysis. With ESI, this approach is widely used for characterizing proteins. LC/MS/MS is also used for protein studies and can be used to determine the amino acid sequence. It is also very useful for drug metabolite studies.

Traceable Quantitative Analysis. Excellent quantitative results can be obtained with these techniques. With an isotope-labeled internal standard, measurement precision is typically 0.5–5%. Accuracy is dependent upon several factors. The measurements must be calibrated

with known mixtures of a pure form of the analyte and the internal standard. Knowledge about the purity of the reference compounds is essential. Other important aspects that must be considered are: liberation of the analyte from the matrix, equilibration with the internal standard, and specificity of the analytical measurements.

Several review articles [4.73–83] are available.

4.1.6 Classical Chemical Methods

Classical chemical analysis comprises gravimetry, titrimetry and coulometry. These techniques are generally applied to assays: analyses in which a major component of the sample is being determined. Classical methods are frequently used in assays of primary standard reagents that are used as calibrants in instrumental techniques. Classical techniques are not generally suited to trace analyses. However, the methods are capable of the highest precision and lowest relative uncertainties of any techniques of chemical analysis. Classical analyses require minimal equipment and capital outlay, but are usually more labor-intensive than instrumental analyses for the corresponding species. Other than rapid tests, such as spot tests, classical techniques are rarely used for qualitative identification.

Kolthoff et al. [4.84] provide a thorough yet concise summary of the classical techniques described in this section.

Gravimetry

Principles of the Technique. Gravimetry is the determination of an analyte (element or species) by measuring the mass of a definite, well-characterized product of a stoichiometric chemical reaction (or reactions) involving that analyte. The product is usually an insoluble solid, though it may be an evolved gas. The solid is generally precipitated from solution and isolated by filtration. Preliminary chemical separation from the sample matrix by ion-exchange chromatography or other methods is often used.

Gravimetric determinations require corrections for any trace residual analyte remaining behind in the sample matrix and any trace impurities in the insoluble product. Instrumental methods, which typically have relatively large uncertainties, can be used to determine these corrections and improve the overall accuracy and measurement reproducibility of the gravimetric analysis, since these corrections represent only a small part of the final value (and its uncertainty).

Scope. Gravimetric determination is normally restricted to analytes that can be quantitatively separated to form a definite product. In such cases, relative expanded uncertainties are in the range of 0.05–0.3%. The applicability of gravimetry can be broadened (at the cost of increased method uncertainty) to cases where the product is a compound with greater solubility and/or more impurities by judicious use of instrumental techniques to determine and correct for residual analyte in the solution and/or impurities in the precipitate. Gravimetry can be labor-intensive and is usually applied to an analytical set of 12 or fewer samples, including blanks.

The advantage of coupling gravimetry with instrumental determination of a residual analyte and contaminants can be demonstrated by the gravimetric determination of sulfate in a solution of K_2SO_4. In an application of this determination [4.85], sulfate was precipitated from a K_2SO_4 solution and weighed as $BaSO_4$. The uncorrected gravimetric result for sulfate was 1001.8 mg/kg with a standard deviation of the mean of 0.32 mg/kg. When instrumentally determined corrections were applied to each individual sample, the corrected mean result was 1003.8 mg/kg sulfate with a standard deviation of the mean of 0.18 mg/kg. The uncorrected gravimetric determination had a significantly negative bias (0.2%, relative) and a measurement reproducibility that was nearly twice that of the corrected result.

The insoluble product of the gravimetric determination can be separated from the sample matrix in several ways. After any required dissolution of the sample matrix, the analyte of interest can be separated from solution by precipitation, ion-exchange or electrodeposition. Other separation techniques (such as distillation or gas evolution [4.86]) can also be used. The specificity of the separation procedure for the analyte of interest and the availability of suitable complementary instrumental techniques will determine the applicability of a given gravimetric determination.

Separation by precipitation from solution can be accomplished by evaporation of the solution, addition of a precipitating reagent, or by changing the solution pH. After its formation, the precipitate may be filtered, rinsed and/or heated and weighed. The resulting precipitate must be relatively free from coprecipitated impurities. An example is the determination of silicon in soil [4.87]. Silicon is separated from a dissolved soil sample by dehydration with HCl and is filtered from the solution. The SiO_2 precipitate is heated and weighed. HF is added to volatilize the SiO_2 and the mass of the remaining impurities is determined. The

SiO_2 is determined by difference. Corrections for Si in the filtered solution can be determined by instrumental techniques.

With ion-exchange separation of the analyte, the precipitation and filtration step may not be required. After collection of the eluate fraction containing the analyte, if no further precipitation reactions are required, the solution can be evaporated to dryness with or without adding other reagents. Ion-exchange and gravimetric determination is demonstrated by the determination of Na in human serum [4.88]. The Na fraction in a dissolved serum sample is eluted from an ion-exchange column. After H_2SO_4 is added, the Na fraction is evaporated to dryness, heated, and weighed as Na_2SO_4. Instrumentally determined corrections are made both for Na in the fractions collected before and after the *Na fraction* and also for impurities in the precipitate.

Electrodeposition can be used to separate a metal from solution. The determination of Cu in an alloy can be used as an illustration [4.86]. Copper in a solution of the dissolved metal is plated onto a Pt gauze electrode by electrolytic deposition. The Cu-plated electrode is weighed and the plated Cu is stripped in an acid solution. The cleaned electrode is reweighed so that the Cu is determined by difference. Corrections are made via instrumental determination of residual Cu in the original solution that did not plate onto the electrode and metal impurities stripped from the Cu-plated electrode.

Nature of the Sample. Samples analyzed by gravimetry must be in solution prior to any required separation. Generally, an amount of analyte that will result in a final product weighing at least 100 mg (to minimize the uncertainty of the mass determination) to no more than 500 mg (to minimize occlusion of impurities) is preferred. Potentially significant interfering substances should be present at insignificant levels. Examples of significant interfering substances would be more than trace B in a sample for a determination of Si (B will volatilize with HF and bias results high) or significant amounts of a substance that is not easily separated from the analyte of interest (for example, Na may not be easily separated by ion exchange from a Li matrix). The suitability of the gravimetric quantification can be evaluated by analyzing a certified reference material (CRM) with a similar matrix using the identical procedure.

Qualitative Analysis. Many of the classical qualitative tests for elements or anions use the same precipitate-forming reactions applied in gravimetry. Gravimetry can also be used to determine the total mass of salt dissolved in a solution (for example, by evaporation with weighing).

Traceable Quantitative Analysis. The mass fraction of the analyte in a gravimetric determination is measured by weighing a sample and the separated compound of known stoichiometry from that sample on a balance that is traceable to the kilogram. Appropriate ratios of atomic weights (gravimetric factors) are applied to convert the compound mass to the mass of the analyte or species of interest. Gravimetry is an absolute method that does not require reference standards. Thus it is considered a direct primary reference measurement procedure [4.89]. Gravimetry can be performed in such a way that its operation is completely understood, and all significant sources of error in the measurement process can be evaluated and expressed in SI units together with a complete uncertainty budget. Any instrumentally determined corrections for residual analyte in the solution or for impurities in the precipitate must rely on standards that are traceable to the SI for calibration. To the extent that the gravimetric measurement is dependent on instrumentally-determined corrections, its absolute nature is debatable.

Titrimetry

Principles of the Technique. The fundamental basis of titrimetry is the stoichiometry of the chemical reaction that forms the basis for the given titration. The analyte reacts with the titrant according to the stoichiometric ratio defined by the corresponding chemical equation. The equivalence point corresponds to the point at which the ratio of titrant added to the analyte originally present (each expressed as an amount of substance) equals the stoichiometric ratio of the titrant to the analyte defined by the chemical equation.

The endpoint (the practical determination of the equivalence point) is obtained using visual indicators or instrumental techniques. Visual indicators react with the added titrant at the endpoint, yielding a product of a different color. Hence, a bias (indicator error) exists with the use of indicators, since the reaction of the indicator also consumes titrant. This bias is evaluated (along with interferent impurities in the sample solvent) in a blank titration. Potentiometric detection generally locates the endpoint as the point at which the second derivative of the potential versus the added titrant function equals zero. Other techniques (amperometry, nephelometry, spectrophotometry, and so on) are also used.

The titrant is typically added as a solution of the given reagent. Solutions are inherently homogeneous and can be conveniently added in aliquots that are not restricted by particle size. The amount of titrant added is obtained from the amount-of-substance concentration (hereafter denoted *concentration*) of the titrant in this solution and its volume (amount-of-substance content and mass, respectively, for gravimetric titrations). The concentration of the solution is obtained by direct knowledge of the assay of the reagent used to prepare the solution, or, more frequently, by standardization. In titrimetry, standardization is the assignment of a value (concentration or amount-of-substance content) to the titrant solution via titration(s) against a traceable standard.

Scope. Titrimetry is restricted to analytes that react with a titrant according to a strict stoichiometric relationship. A systematic bias in the result arises from any deviation from the theoretical stoichiometry or from the presence of any other species that reacts with the titrant. The selectivity of titrimetric analyses is generally not as great as that of element-specific instrumental techniques. However, titrimetric techniques can often distinguish among different oxidation states of a given element, affording information on speciation that is not accessible by element-specific instrumental techniques. Titrimetric methods generally have lower throughput than instrumental methods.

The most commonly encountered types of titrations are acid–base (acidimetric), oxidation–reduction, precipitation, and complexiметric titrations. The theory and practice of each are presented in [4.84]. A detailed monograph [4.90] provides exhaustive information, including properties of unusual titrants.

Titrimetric methods generally have lower throughput than instrumental methods.

Nature of the Sample. Samples analyzed by titrimetry must be in solution or dissolve totally during the course of the given titration. Certain analyses require pretreatment of the sample prior to the titrimetric determination itself. Nonquantitative recovery associated with such pretreatment must be taken into account when evaluating the uncertainty of the method. Examples include the determination of protein using the Kjeldahl titration and oxidation–reduction titrimetry preceded by reduction in a Jones reductor. If possible, a certified reference material (CRM) with a similar matrix should be carried through the entire procedure, including the sample pretreatment, to evaluate its quantitativeness.

Qualitative Analysis. Titrimetry is not generally used for qualitative analysis. It is occasionally used for semiquantitative estimations (for example, home water hardness tests).

Traceable Quantitative Analysis. Titrimetry is considered a primary ratio measurement [4.89], since the measurement itself yields a ratio (of concentrations or amount-of-substance contents). The result is obtained from this ratio by reference to a standard of the same kind. However, titrimetry is different from instrumental ratio primary reference measurements (as in isotope dilution mass spectrometry), in that the standard can be a different element or compound from the analyte. This ability to *link* different chemical standards has been proposed as a basis for interrelating many widely-used primary standard reagents [4.91].

The traceability of titrimetric analyses is based on the traceability to the SI of the standard used to standardize or prepare the titrant. CRMs used as standards in titrimetry are certified by an absolute technique, most often coulometry (see the following section).

Literature references frequently note that certain titrants can be prepared directly from a given reagent without standardization. Such statements are based on historic experience with the given reagent. Traceability for such a titrant rests solely on the manufacturer's assay claim for the given batch of reagent, unless the titrant solution is directly prepared from the corresponding CRM or prepared from the commercial reagent and subsequently standardized versus a suitable CRM. Titrants noted in the literature as requiring standardization (such as sodium hydroxide) have lower and/or variable assays. Within- and between-lot variations of the assays of such reagents are too great for use as titrants without standardization.

The stability and homogeneity of the titrant affect the uncertainty of any titrimetric method in which it is used. The concentration of any titrant solution can change through evaporation of the solvent. A mass log of the solution in its container (recorded before and after each period of storage, typically days or longer) is useful for estimating this effect. In addition, the titrant solution can react during storage, either with components of the atmosphere (for example, O_2 with reducing titrants or CO_2 with hydroxide solutions) or with the storage container (for instance, hydroxide solutions with soda-lime glass or oxidizing titrants with some plastics). Each such reaction must be estimated quantitatively to obtain a valid uncertainty estimate for the given titrimetric analysis.

In titrations requiring ultimate accuracy, the bulk (typically 95–99%) of the titrant required to reach the endpoint is often added as a concentrated solution or as the solid titrant (see the Gravimetric Titrations section). The remaining 1–5% of the titrant is then added as a dilute solution. This approach permits optimal determination of the endpoint, which is further sharpened by virtue of the decreased total volume of solution. Using this approach, a precision on the order of 0.005% can be readily achieved.

Gravimetric Titrations. In traditional gravimetric titrations (formerly called *weight titrations*), the titrant solution is prepared on an amount-of-substance content (moles/kg) basis. The solution is added as a known mass. The amount of titrant added is calculated from its mass and amount-of-substance content.

Gravimetric titrimetry conveys the advantages of mass measurements to titrimetry. Masses are readily measured to an accuracy of 0.001%. Mass measurements are independent of temperature (neglecting the small change in the correction for air buoyancy resulting from the change in air density). The expansion coefficient of the solution ($\approx 0.01\,\%/\mathrm{K}$ for aqueous solutions) does not affect mass measurements.

A useful variation of the dual-concentration approach described above is to add the bulk of the titrant gravimetrically as the solid (provided the solid titrant has demonstrated homogeneity, as in a CRM) or as its concentrated solution. This bulk addition is followed by volumetric additions of the remainder of the titrant (for example, 5% of the total) as a dilute solution for the endpoint determination. The main advantage of this approach is that the endpoint determination can be performed using a commercial titrator. The advantages of gravimetric titrimetry and the dual-concentration approach described above are each preserved. Any effect of variation in the concentration of the dilute titrant is reduced by the reciprocal of the fraction represented by its addition (for example, a 20-fold reduction for 5%).

Coulometry

Principles of the Technique. Coulometry is based on Faraday's Laws of Electrolysis, which relate the charge passed through an electrode to the amount of analyte that has reacted. The amount-of-substance content of the analyte, ν_{analyte}, is calculated directly from the current I passing through the electrode; the time t; the stoichiometric ratio of electrons to analyte n; the Faraday constant F; and the mass of sample m_{sample}. The limits of integration, t_0 and t_{f}, depend on the type of coulometric analysis (see below).

$$\nu_{\mathrm{analyte}} = \frac{\int_{t_0}^{t_{\mathrm{f}}} I\,\mathrm{d}t}{n F m_{\mathrm{sample}}} \tag{4.11}$$

Since I and t can be measured more accurately than any chemical quantity, coulometry is capable of the smallest uncertainty and highest precision of all chemical analyses.

Coulometric analyses are performed in an electrochemical (coulometric) cell. The coulometric cell has two main compartments. The sample is introduced into the sample compartment, which contains the working (coulometric) electrode. The other main compartment contains the counter-electrode. These main compartments are connected via one or more intermediate compartment(s) in series, providing an electrolytic link between the main compartments. The contents of the intermediate compartments may be rinsed or flushed back into the sample compartment to return any sample or titrant that has left the sample compartment during the titration.

Coulometry has two main variants, controlled-current and controlled-potential coulometry. Controlled-current coulometry is essentially titrimetry with electrochemical generation of the titrant. Increments of charge are added at one or more values of constant current. In practice, a small amount of the analyte is added to the cell initially. This analyte is titrated prior to introducing the actual sample. The endpoint of this pretitration yields the time t_0 in (4.11). The quantity t_{f} corresponds to the endpoint of the subsequent titration of the analyzed sample. The majority of the sample (typically 99.9%) is titrated at a high, accurately controlled constant current I_{main} (typically 0.1–0.2 A) for a time t_{main}. Lower values of constant current (1–10 mA) are used in the pretitration and in the endpoint determination of the sample titration. This practice corresponds to the dual-concentration approach used in high-accuracy titrimetry.

Controlled-potential coulometry is based on exhaustive electrolysis of the analyte. A potentiostat maintains the potential of the working electrode in the sample compartment at a constant potential with respect to a reference electrode. Electrolysis is initiated at $t = t_0$. The analyte reacts directly at the electrode at a mass-transport-limited rate. The electrode current I decays exponentially as the analysis proceeds, approaching zero as t approaches infinity. In practice, the electrolysis is discontinued at $t = t_{\mathrm{f}}$, when the current decays

to an insignificant value. A blank analysis is performed without added analyte to correct for any reduction of impurities in the electrolyte.

A survey of coulometric methods and practice through 1986 has been published [4.92].

Scope. Controlled-current coulometry is an absolute technique capable of extreme precision and low uncertainty. Analyses reproducible to better than 0.002% (relative standard deviation) with relative expanded uncertainties of $< 0.01\%$ are readily achieved. Standardization of the titrant is not required. Most of the acidimetric, oxidation–reduction, precipitation, and complexiometric titrations used in titrimetry can be performed using controlled-current coulometry. Compared to titrimetry, controlled-current coulometry has the advantage that the titrant is generated and used virtually immediately. This feature avoids the changes in concentration during storage and use of the titrant that can occur in conventional titrimetry.

Controlled-potential coulometry is also an absolute technique. However, in most cases the correction for the background current limits the uncertainty to roughly 0.1%. Controlled-potential coulometry can afford greater selectivity, through appropriate selection of the electrode potential, than either controlled-current coulometry or titrimetry. The analyte must react directly at the electrode, in contrast to controlled-current coulometry or titrimetry, which can determine nonelectroactive species that react with the given titrant.

Compared to titrimetry, both coulometric techniques have lower throughput. A single high-precision controlled-current coulometric titration requires at least an hour to complete, using typical currents and sample masses. The exhaustive electrolysis required in controlled-potential coulometry requires a period of up to 24 h for a single analysis.

Coulometric techniques are well-suited to automation. Automated versions of controlled-current coulometry [4.93, 94] are used to certify the CRMs used as primary standards in titrimetry.

Nature of the Sample. Sample restrictions for coulometry are similar to those of titrimetry noted above. Additionally, electroactive components other than the analyte can interfere with coulometric analyses, even though the corresponding titrimetric analysis may be feasible.

Qualitative Analysis. Coulometric techniques are not used for qualitative analysis.

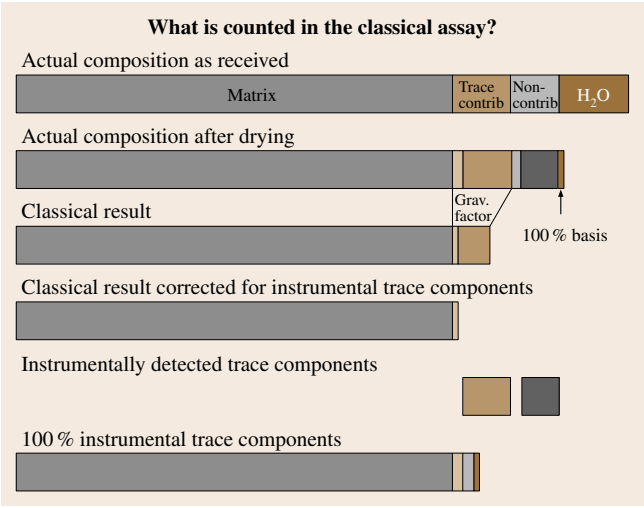

Fig. 4.7 Assay and purity determinations of analytical reagents

Traceable Quantitative Analysis. Traceability for coulometric analyses rests on the traceability of the measured physical quantities I and t and on the universal constant F. In addition, the net coulometric reaction relating electrons to analyte must proceed with 100% current efficiency, so that each mole of electrons that passes through the electrode must react, directly or indirectly, with exactly $1/n$ moles (according to (4.11)) of the added analyte. Interferents or side-reactions that consume electrons yield a systematic bias in the coulometric result. Such interferents must be excluded or taken into account in the uncertainty analysis. The criterion of 100% current efficiency is evaluated by performing trial titrations with different current densities. In controlled-current titrations using an added titrant-forming reagent, its concentration can also be varied to evaluate the generation reaction [4.95].

Coulometry yields results directly as ν_{analyte}, as shown in (4.11). Results recalculated from ν_{analyte} to a mass fraction basis (% assay) must take into account the uncertainty in the IUPAC atomic weights [4.96] in the uncertainty analysis. In high-precision, controlled-current coulometry, this contribution to the combined uncertainty can be significant.

Assay and Purity Determinations of Analytical Reagents

The purity of an analytical reagent can be determined by two different approaches: direct assay of the matrix species, and purity determination by subtraction of trace impurities. In the first approach, a classical assay

technique directly determines the mass fraction of the matrix species. In the second approach, the sum of the mass fractions of all components of the sample is taken as exactly unity (100%). Trace-level impurities in the reagent are determined using one or more of the instrumental techniques described in this chapter. The sum of the mass fractions of all detected impurities is subtracted from 100% to yield the quoted purity (species that are not detected are taken as present at a mass fraction equal to half the detection limit for the given species, with a relative uncertainty of ±100%; the corresponding value and uncertainty are included in the calculation of the purity and its uncertainty). No assay is performed per se.

In principle, both approaches yield the same result. However, difficulties arise in practice owing to the shortcomings of each. In the classical assay, trace impurities can contribute to the given assay, yielding a result greater than the true value. For example, trace Br^- is typically titrated along with the matrix Cl^- in classical titrimetric and coulometric assay procedures. Such trace interferents contribute to the apparent assay to an extent given by the actual mass fraction multiplied by the ratio of the equivalent weight [4.84,90] of the matrix species to that of the actual interferent, analogous to the gravimetric factor in gravimetry. The equivalent weight is given by the molar mass of the given species (calculated from IUPAC atomic weights [4.96]) divided by an integer n. For coulometry, n is that given in (4.11). For other methods, n is defined by the reaction that occurs in the titration or precipitation process.

In the 100% *minus impurities* approach, the result only includes those species that are actually sought. For example, commercial high-purity reagents often state a high purity, such as 99.999%, based on a semiquantitative survey of trace metal impurities. Other species, notably occluded water in high-purity crystalline salts, or dissolved gases in high-purity metals, are not sought, but they may be present at high levels, such as 0.1%. The purity with respect to the stated impurities is valid. However, if the level of unaccounted-for impurities is significant in comparison to the requirements for the calibrant, the stated purity is not valid for use of the reagent as a calibrant.

Figure 4.7 illustrates schematically the contrasting advantages and disadvantages of both approaches toward the purity of a hypothetical crystalline compound. The total length of each bar corresponds to the purity as obtained by the stated method(s). The matrix compound is shown at the left in gray. Impurities (including water) are denoted by segments of other shades at the right end of each bar. The upper bar shows the true composition as received. The impurities are divided into two classes: those that contribute to the classical assay, and those that do not. The second bar shows the true composition after drying. Each class of impurities is subdivided into a component that is detected instrumentally and one that is not. The two components that are detected instrumentally are shown separately in the second line from the bottom.

The third bar shows the classical assay without any corrections for contributing impurities. The lower bar represents the purity obtained from the 100% *minus impurities* approach. Each value has a positive bias with respect to the true assay, the length of the matrix segment. The gravimetric factor represents the ratio of equivalent weights noted above.

The fourth bar shows the result of the classical assay corrected for instrumentally-determined impurities that also contribute to the classical assay. This bar is closest in length to the true assay, represented by the length of the matrix segment. A small bias remains for impurities that both evade instrumental detection and contribute to the classical assay.

An additional problem with classical assays of ionic compounds is that a single technique generally determines only a component of the matrix compound (such as Cl^- in the assay of KCl by titration with Ag^+). The reported mass fraction of the assay compound is calculated assuming the theoretical stoichiometry. The identity of the counterion (such as K^+ in an argentimetric KCl assay) is assumed.

A more rigorous approach toward a *true* assay of an ionic compound by classical techniques is to perform independent determinations of the matrix components. As an example, K^+ in KCl could be assayed by gravimetry, with Cl^- assayed by titrimetry or coulometry. A rigorous version of each of these assays would include corrections for contributing trace interferences to the respective assays.

Several review articles [4.84–96] are available.

4.2 Microanalytical Chemical Characterization

Establishing the spatial relationships of the chemical constituents of materials requires special methods that build on many of the bulk methods treated in the preceding portion of this chapter. There may be interest in locating the placement of a trace chemical constituent within an engineered structure, or in establishing the extent of chemical alteration of a part taken out of service, or in locating an impurity that is impacting on the performance of a material. When the question of the relative spatial locations of different chemical constituents is at the core of the measurement challenge, methods of chemical characterization that preserve the structures of interest during analysis are critical. Not all bulk analytical methods are suited for surface and/or microanalytical applications, but many are. In the remainder of this chapter, some of the more broadly applicable methods are touched upon, indicating their utility for establishing chemical composition as a function of spatial location, in addition to their use for quantitative analysis.

4.2.1 Analytical Electron Microscopy (AEM)

When a transmission electron microscope (TEM) is equipped with a spectrometer for chemical analysis, it is usually referred to as an *analytical electron microscope*. The two most common chemical analysis techniques employed by far are energy-dispersive x-ray spectrometry (XEDS) and electron energy-loss spectroscopy (EELS). In modern TEMs a field emission electron source is used to generate a nearly monochromatic beam of electrons. The electrons are then accelerated to a user-defined energy, typically in the range of 100–400 keV, and focused onto the sample using a series of magnetic lenses that play an analogous role to the condenser lens in a compound light microscope. After interacting with the sample, the transmitted electrons are formed into a real image using a magnetic objective lens. This real image is then further magnified by a series of magnetic intermediate and projector lenses and recorded using a charged coupled device (CCD) camera.

Principles of the Technique. Images with a spatial resolution near 0.2 nm are routinely produced using this technique. In an alternative mode of operation, the condenser lenses can be used to focus the electron beam into a very small spot (less than 1 nm in diameter) that is rastered over the sample using electrostatic deflection coils. By recording the transmitted intensity at each pixel in the raster, a *scanning transmission electron microscope* (STEM) image can be produced. After a STEM image has been recorded, it can be used to locate features of interest on the sample and the scan coils can then be used to reposition the electron beam with high precision onto each feature for chemical analysis. As the beam electrons are transmitted through the sample, some of them are scattered inelastically and produce atomic excitations. Using an EELS spectrometer, a spectrum of the number of inelastic scatters as a function of energy loss can be produced. Simultaneously, an XEDS spectrometer can be used to measure the energy spectrum of x-rays emitted from the sample as the atoms de-excite. Both of these spectroscopies can provide detailed quantitative information about the chemical structure of the sample with very high spatial resolution.

In many ways EELS and XEDS are complementary techniques, and the limitations of one spectroscopy are often offset by the strengths of the other. Because elements with low atomic number do not fluoresce efficiently, XEDS begins to have difficulty with elements lighter than sodium and is difficult or impossible to use for elements below carbon. In contrast, EELS is very efficient at detecting light elements. Because EELS has much better energy resolution than XEDS (≈ 1 eV for EELS and 130 eV for XEDS), it is also capable of extracting limited information about the bonding and valence state of the atoms in the analysis region. The two main drawbacks to EELS are that the samples need to be very thin compared to XEDS samples, and that it places greater demands on the analyst, both experimentally during spectrum acquisition and theoretically during interpretation of the results. Because XEDS works well on relatively thick samples and is easier to execute, it enjoys widespread use, while EELS is often considered a more specialized technique.

Nature of the Sample. Perhaps the single most important drawback to AEM is that all samples must be thinned to electron transparency. The maximum acceptable thickness varies with the composition of the sample and the nature of the analysis sought, but in most cases the samples must be less than ≈ 500 nm thick. For quantitative EELS, the samples must be much thinner: a few tens of nanometers thick at most. Another important limitation is that the samples be compatible with the high vacuum environment required by the electron optics. Fortunately, a wide array of sample prepara-

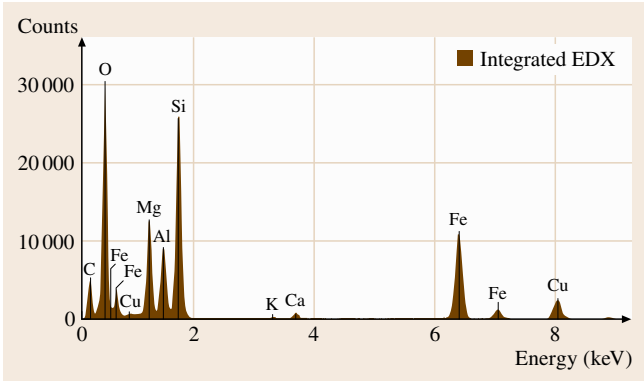

Fig. 4.8 Analytical electron microscopy: example XEDS spectrum from a sample containing C, O, Mg, Al, Si, K, Ca and Fe. The Cu peaks are from the sample mount

tion techniques have been developed over the years to convert macroscopic pieces of (sometimes wet) material into very thin slices suitable for AEM: dimpling, acid jet polishing, bulk ion milling, mechanical polishing, focused ion beam (FIB) processing, and diamond knife sectioning using an ultramicrotome. Preparation of high-quality AEM samples that are representative of the parent material without introducing serious artifacts remains one of the most important tasks facing the AEM analyst.

Qualitative Analysis. The AEM is a powerful tool for the qualitative chemical analysis of nanoscale samples.

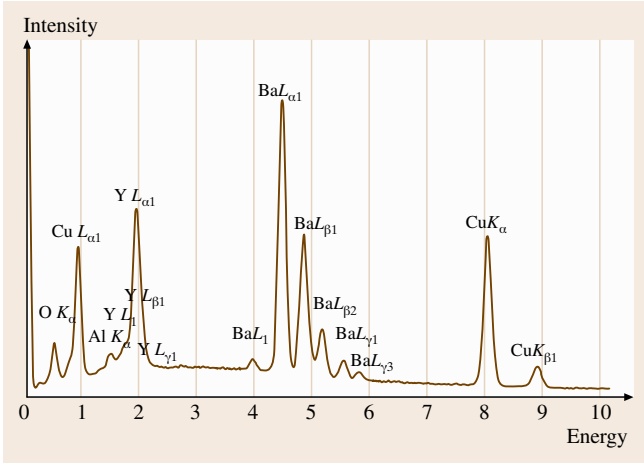

Fig. 4.9 Energy-dispersive spectrometry (EDS) of an $YBa_2Cu_3O_{7-x}$ single crystal with a trace aluminum constituent. Beam energy = 20 keV

The XEDS spectrometer can be used to detect most elements present in the sample at concentrations of 1 mg/g (0.1% mass fraction) or higher (see Fig. 4.8). EELS can be used in many cases down to a detection limit of 100 µg/g, depending on the combination of elements present. While these numbers are not impressive in terms of minimum mass fraction (MMF) sensitivity, it should be noted that this performance is available with spatial resolutions measured in nanometers and for total sample masses measured in attograms. In favorable cases, single-atom sensitivity has been demonstrated in the AEM for several elements, thus establishing it as a leader in minimum detectable mass (MDM) sensitivity.

Traceable Quantitative Analysis. Through the use of standards and the measurement of empirical detector sensitivity factors (Cliff–Lorimer *k*-factors), XEDS measurements in the AEM can be made quantitative. The precision of the measurement is often limited by the total signal available (related to the sample thickness and elemental abundances), while the accuracy is affected by poorly-known sample geometry and absorption effects. Traceability of the results is limited by the extreme rarity of certified reference materials with sufficient spatial homogeneity suitable for the measurement of *k*-factors. EELS measurements can be quantified by a first-principles approach that does not require standards, but this method is limited in practice by our inability to compute accurate scattering cross-sections and our incomplete understanding of solid-state beam–sample interactions.

Several review articles [4.97–100] are available.

4.2.2 Electron Probe X-ray Microanalysis

Most solid matter is characterized on the microscopic scale by a chemically differentiated microstructure with feature dimensions in the micrometer to nanometer range. Many physical, biological and technological processes are controlled on a macroscopic scale by chemical processes that occur on the microscopic scale. The electron probe x-ray microanalyzer (EPMA) is an analytical tool based upon the scanning electron microscope (SEM) that uses a finely focused electron beam to excite the specimen to emit characteristic x-rays. The analyzed region has lateral and depth dimensions ranging from 50 nm to 5 µm, depending upon specimen composition, the initial beam energy, the x-ray photon energy, and the exact analytical conditions.

Table 4.3 Comparison of the characteristics of EDS and WDS x-ray spectrometers

Feature	EDS (semiconductor)	WDS
Energy range	0.1–25 keV (Si) 0.1–100 keV (Ge)	0.1–12 keV (4 crystals)
Resolution at MnK_α	130 eV (Si); 125 eV (Ge)	2–20 eV (E, crystal)
Instantaneous energy coverage	Full range	Resolution, 2–20 eV
Deadtime	50 MS	1 MS
Solid angle (steradian)	0.05–0.2	0.01
Quantum efficiency	≈ 100%, 3–15 keV (Si)	< 30%, variable
Maximum count rate, EDS Maximum count rate, SDD	≈ 3 kHz (best resolution) ≈ 30 kHz (mapping) ≈ 15 kHz (best resolution) ≈ 400 kHz (mapping)	100 kHz (single photon energy)
Full spectrum collection	10–200 s	600–1800 s
Special strengths	Views complete spectrum for qualitative analysis at all locations	Resolves peak interferences; rapid pulses for composition mapping

Principles of the Technique. The EPMA/SEM is capable of quantitatively analyzing major, minor and trace elemental constituents, with the exceptions of H, He and Li, at concentrations as low as a mass fraction of $\approx 10^{-5}$. The technique is generally considered nondestructive and is typically applied to flat, metallographically polished specimens. The SEM permits application of the technique to special cases such as rough surfaces, particles, thin layers on substrates, and unsupported thin layers. Additionally, the SEM provides a full range of morphological imaging and structural crystallography capabilities that enable characterization of topography, surface layers, lateral compositional variations, crystal orientation, and magnetic and electrical fields over the micrometer to nanometer spatial scales. Two different types of x-ray spectrometers are in widespread use, the energy-dispersive spectrometer (EDS) and the wavelength-dispersive (or crystal diffraction) spectrometer (WDS). The characteristics of these spectrometers are such that they are highly complementary: the weaknesses of one are substantially offset by the strengths of the other. Thus, they are often employed together on the same electron beam instrument. The recent emergence of the silicon drift detector (SDD) has extended the EDS output count rate into the range 100–500 kHz. Figure 4.9 shows a typical EDS spectrum from a multicomponent specimen, $YBa_2Cu_3O_7$, demonstrating the wide energy coverage. Figure 4.10 shows a comparison of the EDS and WDS spectra for a portion of the dysprosium L-series. The considerable improvement in the spectral resolution of WDS compared to EDS is readily apparent. Table 4.3 compares a number of the spectral parameters of EDS and WDS.

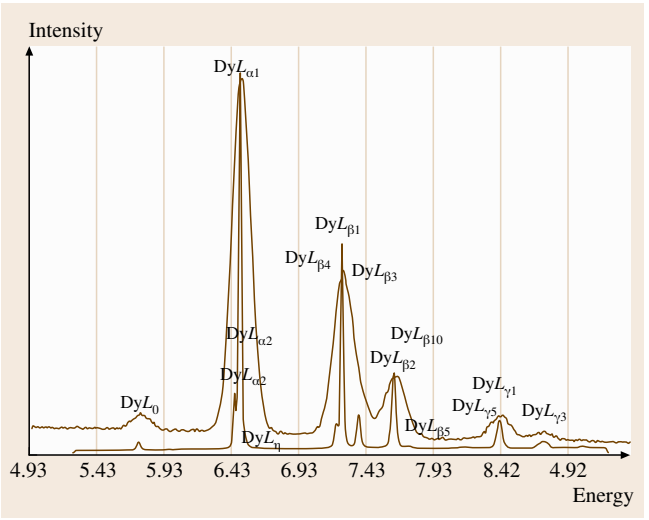

Fig. 4.10 Comparison of EDS and WDS for dysprosium L-family x-rays excited with a beam energy of 20 keV

Qualitative Analysis. Qualitative analysis, the identification of the elements responsible for the characteristic peaks in the spectrum, is generally straightforward for major constituents (for example, those present at concentrations > 0.1% mass fraction), but can be quite challenging for minor (0.01–0.1% mass fraction) and trace constituents (< 0.01% mass fraction). This is especially true for EDS spectrometry when peaks of

minor and trace constituent peaks are in the vicinity (< 100 eV away) of peaks from major constituents. Such interferences require peak deconvolution, especially when the minor or trace element is a light element ($Z < 18$), for which only one peak may be resolvable by EDS. Automatic computer-aided EDS qualitative analyses must always be examined manually for accuracy. The superior spectral resolution of the WDS can generally separate major/minor or major/trace peaks under these conditions, and is also not susceptible to the spectral artifacts of the EDS, such as pile-up peaks and escape peaks. However, additional care must be taken with WDS to avoid incorrectly interpreting higher order reflections ($n = 2, 3, 4, \ldots$ in the Bragg diffraction equation) as peaks arising from other elements.

Quantitative Analysis: Spectral Deconvolution. Quantitative analysis proceeds in three stages:

1. extraction of peak intensities;
2. standardization; and
3. calculation of matrix effects.

For EDS spectrometry, the background is first removed by applying a background model or a mathematical filter. Peak deconvolution is then performed by the method of multiple linear least squares (MLLSQ). MLLSQ requires a model of the peak shape for each element determined on the user's instrument, free from interferences from other constituents. A peak region from an unknown that consists of contributions from two or more constituents is deconvolved by constructing linear combinations of the reference peak shapes for all constituents. The synthesized peaks are compared with the measured spectrum until the best match is obtained, based upon a statistical criterion such as minimization of chi-squared, determined on a channel-by-channel basis. For WDS spectrometry, the resolution is normally adequate to separate the peak interferences so that the only issue is the removal of background. Because the background changes linearly over the narrow energy window of a WDS peak, an accurate background correction can be made by interpolating between two background measurements on either side of the peak.

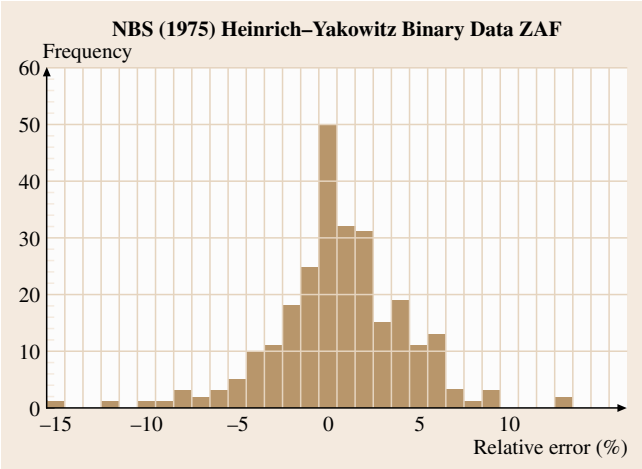

Fig. 4.11 Distribution of analytical relative errors (defined as (100% × [measured − true]/true)) for binary alloys as measured against pure element standards. Matrix correction by National Bureau of Standards ZAF; wavelength-dispersive x-ray spectrometry; measurement precision typically 0.3% relative standard deviation (after Heinrich and Yakowitz)

Quantitative Analysis: Standardization. The basis for accurate quantitative electron probe x-ray microanalysis is the measurement of the ratio of the intensity of the x-ray peak in the unknown to the intensity of that same peak in a standard, with all measurements made for the same beam energy, known electron dose (beam current × time), and spectrometer efficiency. This ratio, known as the k-value, is proportional to the ratio of mass concentrations for the element in the specimen and standard

$$\frac{I_{\mathrm{A,spec}}}{I_{\mathrm{A,std}}} = k \approx \frac{C_{\mathrm{A,spec}}}{C_{\mathrm{A,std}}} \,. \tag{4.12}$$

This standardization step quantitatively eliminates the influence of detector efficiency, and reduces the impact of many physical parameters needed for matrix corrections. A great strength of EPMA is the simplicity of the required standard suite. Pure elements and simple stoichiometric compounds for those elements that are unstable in a vacuum under electron bombardment (such as pyrite, FeS_2 for sulfur) are sufficient. This is a great advantage, since making multielement mixtures that are homogeneous on the micrometer scale is generally difficult due to phase separation.

Quantitative Analysis: Matrix Correction. The relationship between k and $C_{\mathrm{A,spec}}/C_{\mathrm{A,std}}$ is not an equality because of the action of matrix or interelement effects. That is, the presence of element B modifies the intensity of element A as it is generated, propagated and detected. Fortunately, the physical origin of these matrix effects is well-understood, and by a combination of basic physics as well as empirical measurements, multiplicative correction factors for atomic number effects Z, absorption

A and fluorescence F have been developed

$$\frac{C_{\mathrm{A,spec}}}{C_{\mathrm{A,std}}} = kZA\,F\,. \tag{4.13}$$

From the previous discussion, it is obvious that all three matrix effects – Z, A, and F – depend strongly on the composition of the measured specimen, which is the unknown for which we wish to solve. The calculation of matrix effects must therefore proceed in an iterative fashion from an initial estimate of the concentrations to the final calculated value. The measured k-values are used to provide the initial estimate of the specimen composition by setting the concentrations equal to normalized k-values

$$C_{i,1} = \frac{k_i}{\Sigma k_i}\,, \tag{4.14}$$

where i denotes each measured element. The initial concentration values are then used to calculate an initial set of matrix corrections, which in turn are used to calculate predicted k-values. The predicted k-values are compared with the experimental set, and if the values agree within a defined error, the calculation is terminated. Otherwise, the cycle is repeated. Convergence is generally found within three iterations.

This matrix correction procedure has been tested repeatedly over the last 25 years by using various microhomogeneous materials of known composition as test unknowns, including alloys, minerals, stoichiometric binary compounds, and so on. A typical distribution of relative errors (defined as [measured – true]/true × 100%) for binary alloys analyzed against pure element standards is shown in Fig. 4.11.

Compositional Mapping. A powerful method of presenting x-ray microanalysis information is in the form of compositional maps or images that depict the area distribution of the elemental constituents. These maps can be recorded simultaneously with SEM images that provide morphological information [4.101–103]. The digital output from a WDS, EDS or SDD over a defined range of x-ray photon energy corresponding to the peaks of interest is recorded at each picture element (pixel) scanned by the beam. The most sophisticated level of compositional mapping involves collecting a spectrum, or at least a number of spectral intensity windows for each picture element of the scanned image. These spectral data are then processed with the background correction, peak deconvolution, standardization, and matrix correction necessary to achieve quantitative analysis. The resulting maps are actually records of the local concentrations, so that when displayed, the gray or color scale is actually related to the concentration. Figure 4.12 shows examples of compositional maps for an aluminum-nickel alloy.

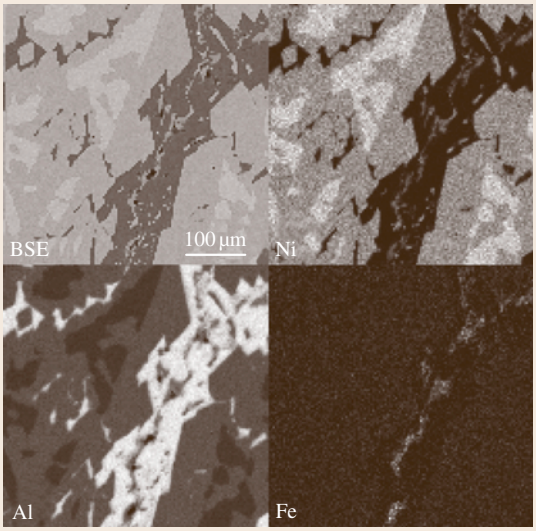

Fig. 4.12 Compositional maps (Ni, Al and Fe) and an SEM image (backscattered electrons, BSE) of Raney nickel (Ni-Al) alloy, showing a complex microstructure with a minor iron constituent segregated in a discontinuous phase

Several review articles [4.104–106] are available.

4.2.3 Scanning Auger Electron Microscopy

Scanning Auger electron microscopy is an electron beam analytical technique based upon the scanning electron microscope. Auger electrons are excited in the specimen by a finely focused electron beam with a lateral spatial resolution of ≈ 2 nm point-to-point in current state-of-the-art instruments. An electron spectrometer capable of measuring the energies of emitted Auger electrons in the range of 1–3000 eV is employed for qualitative and quantitative chemical analysis. As in electron-excited x-ray spectrometry, the positions of the peaks are representative of the chemical composition of the specimen. The inelastic mean free path for Auger electrons is on the order of 0.1–3 nm, which means that only the Auger electrons that are produced within a few nanometers of the specimen surface are responsible for the analytical signal. The current state-of-the-art instruments are capable of providing true surface characterization at ≈ 10 nm lateral resolution.

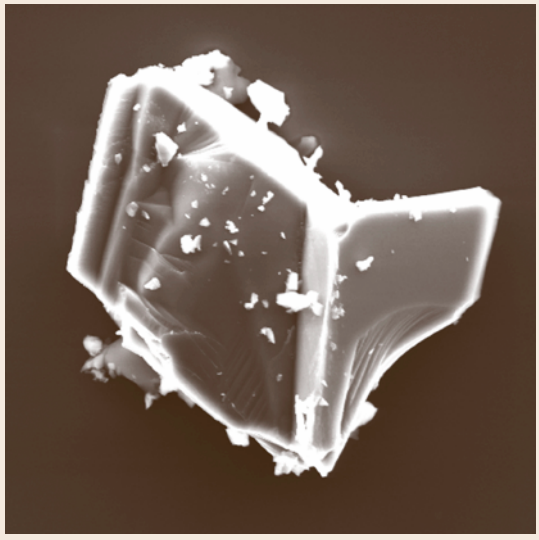

Fig. 4.13 SE image of particle, 25 μm field of view

Principles of the Technique. A primary electron beam interacting with a specimen knocks out a core electron, creating a core level vacancy. As a higher energy level electron moves down to fill the core level vacancy, energy is released in the form of an Auger electron, with the energy corresponding to the difference between the two levels. This is the basis for Auger electron spectroscopy (AES). The core-level vacancy can also be created by an x-ray photon, and this is the basis for x-ray photoelectron spectroscopy. The energy difference between the higher energy electron and the core level can also be released as a characteristic x-ray photon, and this is the basis for electron probe microanalysis.

The primary electron beam in an Auger microscope operates between 0.1 and 30 kV, and beam currents are on the order of nanoamps for analysis. Tungsten and lanthanum hexaboride electron guns can be used for AES, but field emission electron guns are the best choice because of the higher current density. It is desirable for AES to have more electrons in a small spot, and field emission guns deliver the smallest spot sizes normalized to beam current. Auger microscopes are also very good scanning electron microscopes, capable of producing secondary electron images (see Fig. 4.13) of the specimen as well as backscattered electron images if so equipped.

Auger electrons are produced throughout a sample volume defined by the interaction of the primary electron beam and the specimen. Auger electrons are relatively low in energy and so can only travel a small distance in a solid. Only the Auger electrons that are created close to the surface, within a few nanometers, have sufficient mean free path to escape the specimen and be collected for analysis. Since the Auger information only comes from the first few nanometers of the specimen surface, AES is considered a surface-sensitive technique. Several review articles are available.

Nature of the Sample. The surface sensitivity of AES requires the specimen to have a clean surface free of contamination. For this reason, Auger microscopes are ultrahigh vacuum (UHV) in the specimen chamber, which is on the order of 10^{-8} Pa. Steps must be taken to clean specimens prior to introduction into the Auger microscope so that they are free of volatile organic compounds that can contaminate the chamber vacuum. The Auger specimen chamber is equipped with an argon ion gun for sputter cleaning-off the contamination or ox-

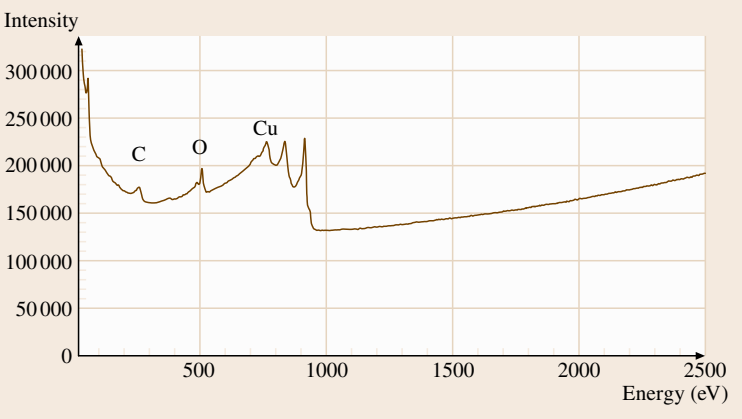

Fig. 4.14 Direct AES of copper with carbon and oxygen

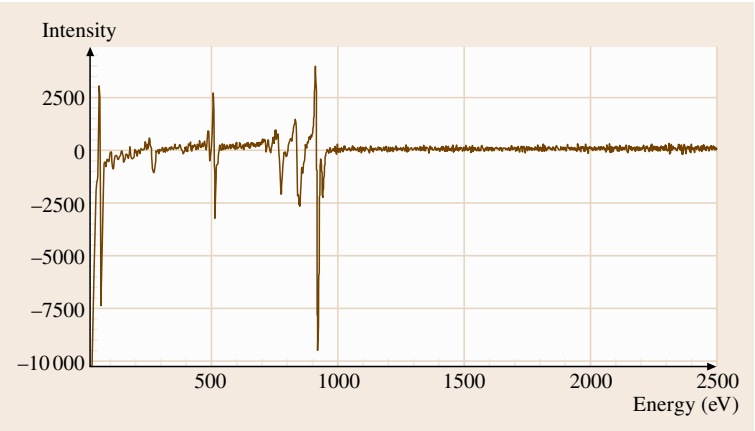

Fig. 4.15 Derivative AES of copper with carbon and oxygen

ide layer that coats specimens as a result of transporting them in air.

Investigation of a buried structure or an interface that is deeper than the Auger escape depth can be accomplished by Auger depth profiling. In Auger depth profiling, the instrument alternates between Ar ion sputtering of the surface and Auger analysis of the surface until, as material is sputtered away, the elemental composition changes with depth.

Qualitative/Quantitative Analysis. Auger electrons are recorded as a function of their energy by the electron spectrometer in the Auger microscope and provide elemental as well as bonding information. There are two types of electron spectrometer, the cylindrical mirror analyzer (CMA) and the hemispherical analyzer (HSA). The CMA is concentric with the electron beam and has a greater throughput because of its favorable solid angle. The HSA has the higher energy resolution, which is desirable for unraveling overlapped peaks. In the direct display mode (Fig. 4.14), peaks on the sloping background of an Auger spectrum indicate the presence of elements between Li and U. Spectra can also be displayed in the derivative mode (Fig. 4.15), which removes the sloping background and random noise. Auger quantitation is complicated by many instrumental factors and is normally done with sensitivity factors normalized to an elemental silver Auger signal collected under the same instrumental conditions.

Several review articles [4.107–109] are available.

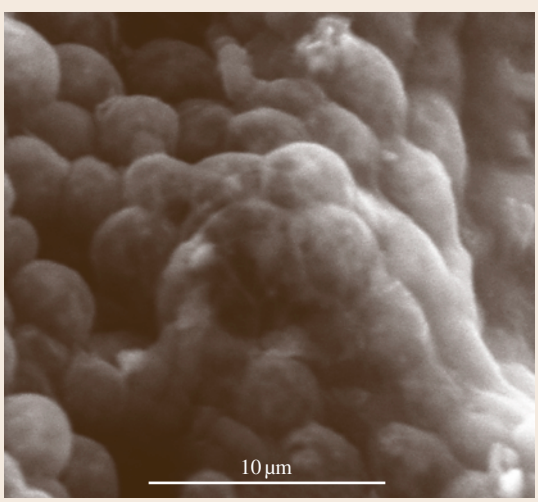

Fig. 4.16 ESEM image of hydrated, freshwater algal surface

4.2.4 Environmental Scanning Electron Microscope

The environmental scanning electron microscope (ESEM) is a unique modification of the conventional scanning electron microscope (SEM). While the SEM operates with a modest vacuum ($\approx 10^{-3}$ Pa), the ESEM is able to operate with gas pressures ranging between 10 and 2700 Pa in the specimen chamber due to a multistage differential pumping system separated by apertures. The relaxed vacuum environment of the ESEM chamber allows examination of wet, oily and dirty specimens that cannot be accommodated in the higher vacuum of a conventional SEM specimen chamber. Perhaps more significant, however, is the ability of the ESEM to maintain liquid water in the specimen chamber with the use of a cooling stage (Fig. 4.16). The capability to provide both morphological and compo-

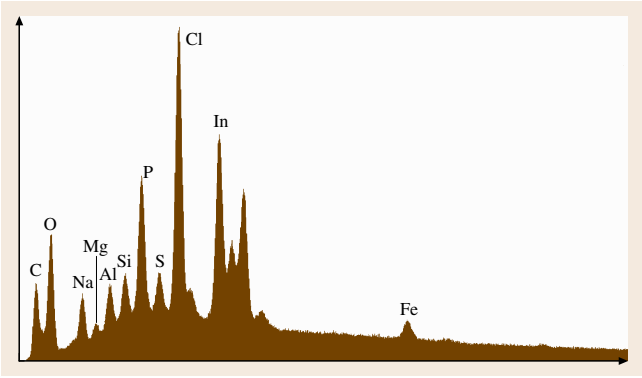

Fig. 4.17 EDS image of biological solids from a wastewater treatment facility. The indium peak is caused by the support stub

sitional analysis of hydrated samples has allowed the ESEM to benefit a number of experimental fields, ranging from material science to biology. Several review articles are available.

Principles of the Technique. The ESEM utilizes a gaseous secondary electron detector (GSED) that takes advantage of the gas molecules in the specimen chamber. The primary electron beam, operating between 10 and 30 kV, are generated from tungsten, lanthanum hexaboride, or field emission electron guns. When a primary electron beam strikes a specimen, it generates both backscattered and secondary electrons. Backscattered electrons are energetic and are collected by a line-of-sight detector. The secondary electrons are low-energy and, as they emerge from the specimen, are accelerated towards the GSED by the electric field set up between the positive bias on the GSED and the grounded specimen stage. These secondary electrons collide with gas molecules, resulting in ionizations and more secondary electrons, which are subsequently accelerated in the field. This amplification process repeats itself multiple times, generating imaging gain in the gas. A byproduct of this process is that the gas molecules are left positively charged and act to discharge the excess electrons that accumulate on an insulating specimen from the primary electron beam. This charge neutralization obviates the need for conductive coatings or low-voltage primary beams, as are often used in conventional SEM to prevent surface charging under the electron beam.

Secondary and backscattered electrons are produced throughout the interaction volume of the specimen, the depth of which is dependent on the energy of the primary electron beam and the specimen composition. The backscattered electrons contain most of the energy of the primary electron beam and can therefore escape from a greater depth in the specimen. In contrast, secondary electrons are only able to escape from the top 10 nm of the specimen, although backscattered electrons can also create secondary electrons prior to exiting the sample and provide sample depth information to the image. In general, it is possible to routinely resolve features ranging from 10 to 50 nm. However, the primary electron beam can also interact with the gas molecules, resulting in beam electrons being scattered out of the focused electron beam into a wide, diffuse skirt that surrounds the primary beam impact point. Similarly, chamber gas composition can also impact the amplification process and thereby affect image quality.

Qualitative/Quantitative Analysis. In addition to the image-producing backscattered and secondary electrons that are generated when a primary beam strikes a specimen, there are also electron beam interactions that result in the generation of x-rays from the interaction volume. The energy of the resulting x-rays is representative of the chemical composition within the interaction volume and can be measured with an EDS. X-ray counts are plotted as a function of their energy, and the resulting peaks can be identified by element and line with standard x-ray energy tables (Fig. 4.17). EDS in the ESEM is considered a qualitative method of compositional analysis since x-rays may originate hundreds of micrometers from the impact point of the primary electron beam as a result of electrons scattered out of the beam by gas molecules.

Several review articles [4.110–117] are available.

4.2.5 Infrared and Raman Microanalysis

Infrared and Raman microanalysis is the application of Raman and/or infrared (IR) spectroscopies to the analysis of microscopic samples or sample areas. These techniques are powerful approaches to the characterization of spatial variations in chemical composition for complex, heterogeneous materials, operating on length scales similar to those accessible to conventional optical microscopy while also yielding the high degree of chemical selectivity that underlies the utility of these vibrational spectroscopies on the macroscale. Sample analyses of this type are particularly useful in establishing correlations between macroscopic performance properties (such as mechanical and chemical stability, biocompatibility) and material microstructure, and are thus a useful ingredient in the rational design of

high-performance materials. Several review articles are available.

Principles of the Technique. A typical Raman microscope comprises a laser excitation source, a light microscope operating in reflection mode, and a spectrometer. Photons inelastically scatter from the sample at frequencies shifted from that of the excitation radiation by the energies of the fundamental vibrational modes of the material, giving rise to the chemical specificity of Raman scattering. A high-quality microscope objective is used both to focus the excitation beam to an area of interest on the sample and to collect the backscattered photons. In general, the Rayleigh (elastic) scattering of the incident photons is many orders of magnitude more efficient than Raman scattering. Consequently, the selective attenuation of the Rayleigh photons is a critical element in the detection scheme; recent advances in dielectric filter technology have simplified this problem considerably. The attainable spatial resolution is, in principle, limited only by diffraction, allowing submicrometer lateral resolution in favorable cases. Fine vertical resolution can also be achieved through the use of a confocal aperture, opening up the possibility of constructing 3-D chemical images through the use of Raman depth profiling. Raman images are usually acquired by raster scanning the sample with synchronized spectral acquisition. Wide-field illumination and imaging configurations have been explored, but they are generally only useful in limited circumstances due to sensitivity issues. Chemical composition maps can easily be extracted from Raman images by plotting the intensities of bands due to particular material components. Subtle spectral changes (such as band shifts) can also be exploited to generate spatial maps of other material properties, such as crystallinity and strain.

A typical IR microscope system consists of a research-grade Fourier transform (FT) IR spectrometer coupled to a microscope that operates in both reflection and transmission modes. Reflective microscope objectives are widely used due to their uniformly high reflectivity across the broad infrared spectral region of interest and their lack of chromatic aberration. The spectrum of IR light measured upon transmission through or reflection from the sample is normalized to a suitable background spectrum. The normalized spectrum displays attenuation of the IR light reaching the detector due to direct absorption at frequencies resonant with the active vibrational modes of the sample components. The frequencies at which absorption occurs are characteristic of the presence of particular functional groups (such as C=O), resulting in the powerful chemical specificity of the measured spectra (Fig. 4.18). Microscopes employing a sample raster scanning approach to image acquisition were the first available, but have now been joined by those employing a wide-field illumination, array-based imaging detection approach. The spatial resolution attainable with this

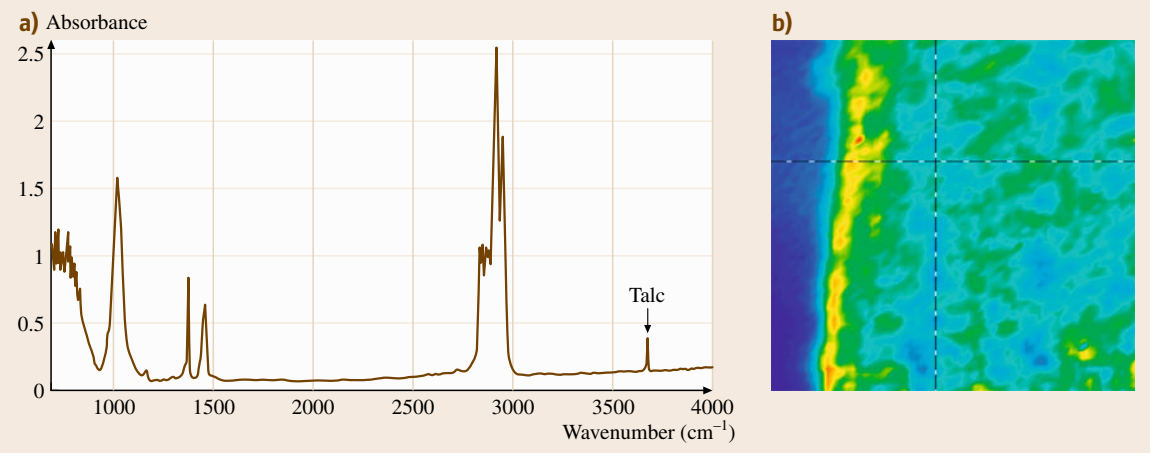

Fig. 4.18 (**a**) IR microspectrum of a thin film microtome section of injection-molded thermoplastic olefin. The sharp spectral feature at 3700 cm^{-1} is due to the OH stretching vibration of the talc filler (**b**) 325 µm × 325 µm IR image of a thermoplastic olefin cross-section wherein the amplitude of the talc band is plotted on a *blue* (low amplitude) to *red* (high amplitude) color scale. The *yellow-red band* on the *left side* of the film is due to a talc-rich layer formed near the mold surface

technique is typically on the order of 20–40 μm, and diffraction-limited performance is not achieved due to source brightness limitations. Several alternative sampling techniques that have found widespread utility in IR spectroscopy of macroscopic samples have been successfully adapted on the microscale, including attenuated total reflection (ATR) and grazing incidence reflectivity. Maps of chemical composition can be extracted from IR spectral images in a manner similar to the Raman case, wherein amplitudes of bands due to material components of interest are plotted as a function of position on the sample.

Raman and IR microanalysis are complementary techniques, as is the case for their macroscopic analogs, widely applicable to extended solids, particles, thin films and even these materials under liquid environments. The choice between these analysis techniques is often dictated by the relative strength of the Raman and/or IR transitions that most effectively discriminate among the sample components. Notably, the molecular properties that dictate the strength of these transitions, molecular polarizability in the case of Raman and transition dipole moments for IR, are not generally correlated. In fact, for centrosymmetric molecules the techniques are particularly complementary as IR transitions forbidden by symmetry are by definition Raman active and vice versa. Sample considerations also play a role in this choice, as the nature of the material can preclude the use of one (or both) techniques. Raman microscopy can be used to study a broad array of materials, as the Raman photons are scattered over a wide, often isotropic, distribution of solid angles and are thus easily detected by the same microscope objective used for excitation. In contrast, transmission IR microscopy requires that the sample of interest be mounted on an IR-transparent substrate and that the sample itself be sufficiently thin to avoid saturation effects. Similarly, reflection mode IR microscopy is optimized when the analyte is mounted on a highly reflective substrate; the constraints on sample thickness apply in this configuration as well. However, Raman microscopy suffers from another significant limitation, as background fluorescence precludes the measurement of high signal-to-noise Raman spectra for many materials, particularly for higher-energy excitation wavelengths (for example, 488 and 532 nm). It is often the case that the shot noise present on a large fluorescence background is sufficiently larger than the Raman signal itself such that no amount of signal averaging will yield a high-quality spectrum. Notably, typical cross sections for fluorescence are vastly larger than those of Raman scattering, so the Raman excitation wavelength need not be in exact resonance with a sample electronic transition to yield an overwhelming fluorescence background. This problem can be mitigated by the use of lower energy excitation wavelengths (for example, 785 and 1064 nm), although the Raman scattering efficiency drops with λ^4. The cross-sections for Raman scattering are generally much lower than those of IR absorption, and so some materials with low Raman cross-sections are not amenable to Raman microanalysis, simply due to lack of signal, particularly in the microanalysis context. Although the Raman signal does scale with incident intensity, sample damage considerations typically limit this quantity. IR microanalysis often suffers from the opposite problem, wherein even microscopic samples can absorb sufficient radiation to lead to saturation effects in the spectra.

Nature of the Sample. The sample preparation requirements for Raman microscopy are quite modest; the surfaces of most solid materials are easily examined and some depth profiling is also possible depending on the material transparency. The Raman spectra of some materials are dominated by a fluorescent background; this is the most important sample property limiting the application of Raman microscopy. Two factors are critical in sample preparation for IR microscopy: choice of mounting substrate and sample thickness. For transmission microscopy, the substrate is limited to a set of materials that are broadly transparent over the IR (such as CaF_2 or KBr). In reflection microscopy, the substrate is often a metal film that is uniformly reflective across the IR region (such as Au or Ag). The issue of sample thickness is related to the onset of saturation effects in the spectra. The cross-sections for many IR absorption transitions are sufficiently large that samples can absorb nearly all of the resonant incident radiation, leading to spectral artifacts that interfere with both qualitative and quantitative analysis. For example, polymers as thin as 30 μm can show saturation artifacts in the C–H stretching bands. Sample preparation methods such as microtomy and alternative sampling methods such as μ-ATR can be used to address this problem for some classes of samples.

Qualitative Analysis. IR and Raman microspectroscopy are both powerful tools for the qualitative analysis of microscopic samples. The appearance of particular bands in the measured vibrational spectra indicate the presence of specific functional groups, and the chemical structure of the analyte can often be obtained from an analysis of the entire spectrum. Additionally, large libraries of IR and Raman spectra of a wide variety of

materials are now available, greatly facilitating the use of spectral matching algorithms in the identification of materials on the basis of the IR and/or Raman spectrum. The chemical specificity of vibrational spectroscopy is undoubtedly its most powerful characteristic, one that is particularly useful in the identification of various components of complex materials that are compositionally heterogeneous on microscopic length scales. The sensitivity of these techniques is difficult to characterize as cross-sections for these types of transitions generally vary over many orders of magnitude and thus the sensitivity for different analytes varies in a similar manner. However, analytes that occupy the minimum focal volumes attainable in these microscopies (Raman: $1\,\mu m \times 1\,\mu m \times 3\,\mu m$, IR: $25\,\mu m \times 25\,\mu m \times 50\,\mu m$) are generally detectable in both IR and Raman (particularly for strong scatterers).

Traceable Quantitative Analysis. Although traceable quantitative analysis of macroscopic samples with IR spectroscopy is well-established (particularly for gases), extension to the microanalysis of solids is quite challenging. Through the use of well-characterized standard materials, IR microscopy can be used for quantitation, although the accuracy of this approach is often limited by optical effects (such as scattering) due to the complex morphology of typical samples. Estimates of Raman scattering cross-sections can be made in favorable cases, based on the characterization of instrumental factors affecting detection efficiency. However, extension to quantitative analysis is impractical due to a lack of reference materials and difficulties associated with the ab initio calculation of Raman cross-sections for all but the simplest materials.

Several review articles [4.118–121] are available.

4.3 Inorganic Analytical Chemistry: Short Surveys of Analytical Bulk Methods

In addition to the description of measurement methods for inorganic chemical bulk characterization (Sect. 4.1) short surveys of analytical methods are summarized in this section, outlining specifications of typical values of sample volume or mass and limits of detection as well as outputs, and relevant examples of applications.

Quite in general, metrology in chemistry and, therefore, in inorganic chemical analysis in special, has its own characteristics and singularities not to be found in this kind or in analogous manner in the field of physical metrology [4.122]. In this context the terms *selectivity* or *qualitative analysis* are essential keywords to characterize the problem. A typical example could arise from spectral interferences, were the characteristic signal of an element A (to be measured) could not only be interfered by that of another element B thus adulterating the result for the mass fraction of the element A, but also a characteristic signal of the element B could be erroneously be interpreted as a characteristic signal of the element A. In other words, in this case one could have tried to measure the mass fraction of e.g. copper in a sample, but in reality he would have measured the mass fraction of iron. Therefore the selectivity of a method is a very important characteristic and may have been one main reason why in the practical everyday work very precise working classical chemical methods of often rather lower selectivity, such as gravimetry, coulometry or titrimetry, were substituted on a large scale by modern methods of higher selectivity step by step in the past, even when the results of letter ones showed much lower precision.

Concerning the traceability of results of inorganic chemical analysis to the SI unit (mol or kg of the relevant analyte) for almost all inorganic analytical methods calibration of the analytical instruments is necessary by using solutions or substances of known content of the analyte. *Known content* means: a known mass fraction or concentration of the analyte – at which the specified value of content must include the specified value of its uncertainty according to GUM based on a definite traceability chain.

If methods needing calibration are used for the *analysis of liquid samples* calibration solutions are prepared from basic (*stock*) solutions which either come from commercial suppliers or are prepared in the laboratory by chemical dissolution of a definite mass of a material of definite purity in elemental form or as a compound of definite stoichiometry and purity (e.g. a pure metal or a pure metal salt, respectively), – or such solutions are only used to verify the elemental concentration of a commercial solution which is used as calibration stock solution after verification of its analyte concentration. After all, in all cases high purity substances with well known content of the main component (the respective element to be determined) are the starting materials for preparation of stock solutions and therefore actual

transfer standards directly to the SI unit (mol or kg of the analyte). Without such materials a really complete traceability chain to the SI unit does not exist, because the uncertainty of the final calibration solution would be based on assumptions about the purity of the starting material. An internationally harmonized system of such primary pure transfer standards directly to SI unit does not exist. First international attempts to harmonize measurement results of purity assessment of high-purity materials were made by national metrological institutes in the frame of CCQM by interlaboratory comparisons for the determination of a limited number of analytes in materials of pure nickel [4.123] and of pure zinc [4.124]. For an example of a national attempt to establish a system of primary pure materials as National Primary Standards for Elemental Analysis and of a traceability system based on those standards (Sect. 4.5).

In case of *direct analysis of solid samples* certified matrix reference materials or other appropriate solid materials of similar composition as the sample to be analyzed having known analyte contents must be used for calibration in the majority of cases. In this situation the traceability chain to the SI unit (mol or kg of the analyte) is less direct than for calibration with methods applied to the analysis of liquid samples. This is, because the process of certification of such matrix materials used for calibration commonly includes the calibration of instruments with liquid calibration samples.

The metrological advantage of methods needing calibration by liquid calibration samples over those needing calibration by solid matrix materials is also based on the fact that liquid samples of homogeneously distributed and definite analyte and matrix concentrations can easily prepared by mixing or diluting of definite volumes or masses of stock solutions of definite concentration of analytes. In most cases an analogy to this fact does not really exist for the direct analysis of solid samples because of problems with losses, contamination or lack of homogeneity when adequate solid calibration samples are prepared. Therefore such solid calibration samples, such as e.g. powder mixtures or samples of metal alloys, normally after their preparation, need a certification of their real mass fractions by either methods not needing a calibration (which normally are not available) or by methods based on instrument calibration with liquids.

As quite explained above, the selectivity of a method is of high importance. The analysis of complex samples can often induce matrix effects as a combination of influences of the other elements in the sample on the characteristic signal of the analyte to be determined or of chemical or physical differences between calibration sample and measured sample (such as acidity of solutions or grain size of powders). Those effects impair the trueness of results and can be decreased by matching of the composition of calibration samples to the composition of the sample to be analyzed (matrix matching, matrix adaptation) or by application of the method of standard addition. However, the method of standard addition (as mainly used with AAS, see below) is based on the precondition that the measured characteristic signal would be zero if the sample would contain no content of the measured analyte. This cannot be ensured in many cases. Internal standardisation can also be used to reduce matrix effects. In this case it must be assumed that the signal of the internal standard elements reacts with the same quantitative change to matrix influences as the signal of the investigated analyte. However, also this is not always the case.

4.3.1 Inorganic Mass Spectrometry

Today this field is dominated by inductively coupled plasma mass spectrometry (ICP-MS) and by glow discharge mass spectrometry (GD-MS). ICP-MS is mainly used for the analysis of liquid samples while GD-MS is used for direct analysis of solid samples.

ICP–MS

ICP-MS combines the advantages of extremely low detection limits, broad multielement-capability – and (especially in case of using high resolution mass spectrometers) a relatively low number of serious spectral interferences in comparison to ICP OES. ICP-MS spectrometers are latterly combined with chromatographs, especially with GC- or HPLC-chromatographs, to measure the contents of organometallic compounds in the frame of speciation analysis. Thus the high selectivity of chromatography for such species is combined with the extremely high detection power of ICP-MS not to achieve by only using combined methods of organic chemical analysis, such as GC-MS or HPLC-MS. But in the approach of the analysis with ICP-MS, such methods are often used to identify the compounds belonging to the chromatographic peaks.

Isotope Dilution

Isotope dilution in combination with mass spectrometry (TIMS or ICP-MS) offers an enormous advantage because the internal standardization is achieved using the same chemical element as the measured one. Moreover,

there is the advantage, too, of eliminating adulterations of the results arising in the process of chemical sample preparation if the spike can be added to the sample before chemical treatment of the sample. However, the metrological precondition of the highly preferable method of isotope dilution is always the knowledge of the purity of the isotope spike used concerning its total mass fraction of the relevant element.

GD-MS

GD-MS instruments though offering the advantage of rather fast direct solid sample analysis are much less in use than ICP-MS instruments. GD-MS can be powered by either a direct-current (DC) or radiofrequency (RF) power supply. Up to now the letter option is not available in commercially offered GD-MS instruments, although RF instruments have the advantage that not only electrically conducting but also nonconducting materials can be directly be analyzed. In the past there had been only one commercial GD-MS instrument widespread among a larger number of users, – this was the VG-9000 (Thermo Elemental, UK). Several instruments of this type are still in use. The VG 9000 has not been produced since some years since it was replaced by the new Element GD (Thermo Instr. Corp., USA) which is a double focussing high resolution spectrometer, too. But its GD cell is based on a Grimm type geometry allowing faster sputtering than with the GD

Table 4.4 Inorganic mass spectrometry

Analytical method	Inorganic mass spectroscopy (MS) TIMS, ICP-MS, GD-MS, SS-MS
Measurement principle	Measurement of the mass spectra of ions generated due to the ionisation in an ionization source (thermal-ionization *TI*, inductively coupled plasma *ICP*, glow discharge *GD*, or electrical spark source *SS*; – ICP-MS sometimes combined with laser ablation). A mass spectrum consists of peaks of ions of a definite ratio of their atomic masses divided by their charge number (m/z). It results from a stream of gaseous ions with different values of m/z. The intensity of the spectral mass peaks of isotopes corresponds with the concentration of the corresponding element in the plasma
Sample volume/mass	*TIMS*: wide range depending on sample preparation, (final sub-sample 1–10 µL); *ICP-MS*: 1–10 mL, *GD-MS* and *SS-MS* (direct solid sampling methods): ablated sample mass small, depending on instrument and parameters used: about 1–20 mg
Typical limits of detection	*TIMS*: pg to ng – absolute (upper ng kg^{-1} to lower µg kg^{-1} range relative) *ICP-MS*: 0.001–0.1 µg L^{-1} (using high resolution spectrometers and in aqueous solution up to 2 orders of magnitude lower) GDMS, SSMS: 0.1–10 µg kg^{-1}
Output, results	Simultaneous and sequential multielement analyses *TIMS*: high precision, high accuracy, highest metrological level with isotope dilution technique (*IDMS*), but raw analyte isolation needed. Advantages of *ICP-MS*: Simultaneous multielement capability, very low limits of detection, very high dynamic range. Combined with laser ablation (*LA ICP-MS*): for solid samples; combined with isotope dilution technique (*ID-ICP-MS*): high metrological level results of high accuracy *GD-MS*: Simultaneous multielement capability, direct sampling without chemical sample handling, very low detection limits, very high dynamic range *SS-MS*: Not yet widely-used
Typical applications	Qualitative and quantitative elemental and isotopic analysis, e.g. semiconductors, ceramics, pure metals, environmental and biological materials, high purity reagents, nuclear materials, geological samples; Speciation analysis when *ICP-MS* is combined with chromatographic methods *ID-MS*: Metrological high level technique for reference values of international comparisons in CCQM and for certification of reference materials

cell of VG 9000 and therefore a distinctly shortened time for one analysis. The Element GD is now getting its global acceptance. GD-MS can also be used for the determination of several nonmetals. This is of special interest because only few methods can be used for this important task. The detection limits can be considerably decreased when mixtures of argon and helium are used instead of argon alone as working gas of GD as it was demonstrated for trace determination of different nonmetals in pure copper samples [4.125].

GD-MS calibration is normally based on the availability of appropriate reference materials which may be a very restricting condition for carrying out quantitative analyses by this method. Because of lack of appropriate reference materials this restriction is especially unfavourable in case of ultra-trace determination. An alternative possibility to achieve a calibration having a shorter traceability chain and being applicable without having appropriate reference materials available was demonstrated in case of analysis of ultra-pure metals using calibration samples made from pure metal powders quantitatively doped with calibration liquids and pressed to pellets under high pressure after drying and homogenization [4.126].

Some relevant information concerning the inorganic mass spectrometry is summarized in Table 4.4.

4.3.2 Optical Atomic Spectrometry

Atomic absorption spectrometry (AAS) is a method of high selectivity mainly applied to the analysis of liquids. The Flame AAS is very robust and still applied in many laboratories although it is increasingly substituted by ICP OES. One reason is the mono-elemental character of one measurement cycle in AAS needing a time consuming change of element-specific radiation source (such as hollow cathode lamp or electrodeless discharge lamp) when another element shall be measured. In the modern high resolution continuum source AAS (HR-CS AAS) [4.127] only one continuous radiation source is used for all elements to be determined in a spectral region of 190–900 nm. The graphite furnace AAS is a very sensitive micro-method. In recent times specific AAS spectrometers for direct electro-thermal evaporation of solid micro-samples are also available. The powdered micro-sample is directly weighed into the sample boat of the graphite furnace of the instrument.

ICP optical emission spectrometry (ICP OES) is now the most widespread method for the multielement determination of liquid samples. The method is very robust. Most instruments contain compact Echelle-spectrometers with area detectors such as CCD or CID; – or classical spectrometer types (monochromators or polychromators) are used combined with line detectors or PMTs. ICP OES can be combined with devices for electrothermal vaporization of solid micro-samples. In several cases and after checking this possibility, the instrument can even be calibrated by using solutions, thus enabling a short traceability chain to SI unit as demonstrated e.g. in case of analysis of plant materials [4.128].

The spark OES [4.129] is since many years worldwide widespread and the workhorse in metallurgical industry, especially for the direct determination of traces and minor components in production control and in final check of composition of metals and alloys. The method is extremely fast and robust and has the advantage that micro-inhomogeneity of the sample in the spark spot is largely compensated during the pre-spark phase by micro-melting action of many single individual sparks.

The glow discharge OES (GD-OES) can be alternatively applied to the spark-OES for the bulk analysis of metals. It has in some cases the advantage of a higher trueness of results, but it is often less robust and not so fast as the spark-OES. The main area of application of GD-OES is the analysis of layers or surfaces of electrically conducting and (in case of RF-GD cells) also of non-conducting samples.

Some relevant information concerning the optical atomic spectrometry is summarized in Table 4.5 for AAS and in Table 4.6 for OES.

4.3.3 X-ray Fluorescence Spectrometry (XRF)

X-ray fluorescence spectrometry (XRF) in its classical form is an important method used for the direct analysis of solid samples. It is not really a trace method but its applicability reaches from determination of higher trace contents up to the precise measurement of the main matrix component. Depending on counting rate and time the precision of the method can be extremely high; – but it is necessary to use very well matrix matched calibration materials to achieve a high trueness of the results, too. In metallurgical industry XRF and spark-OES are complementary mutually. XRF has the advantage that electrically non-conducting materials can also be analyzed. If direct solid sample technique is used, traceability is achieved via calibration with certified matrix reference materials of a similar composition as the samples to be analyzed. If the borate fusion technique is used, calibration samples can be

Table 4.5 Atomic absorption spectrometry

Analytical method	Atomic absorption spectrometry (AAS) F AAS, GF AAS (ET AAS), HG AAS, SS ET AAS
Measurement principle	Measurement of the optical absorption spectra of atoms based on the absorption of radiation (emitted by a primary (background) radiation source) by the atoms (being mainly) in the ground state in the gaseous volume of the atomized sample. The strength of the line absorption corresponds with the concentration of the element in the absorbing gaseous volume
Sample volume/mass	Flame AAS (*F AAS*): 1–10 mL graphite furnace (*GF AAS*) (= electro thermal atomisation AAS, ET AAS): 0.01–0.1 mL hydride generation AAS (*HG AAS*): 0.5–5 mL solid sampling (*SS ET AAS*): 0.1–50 mg
Typical limits of detection	*F AAS*: 1–1000 $\mu g\,L^{-1}$, *GF AAS*: 0.01–1 $\mu g\,L^{-1}$, *HG AAS*: 0.01–0.5 $\mu g\,L^{-1}$, *SS ET AAS* as direct solid sampling method: 0.01–100 $\mu g\,kg^{-1}$
Output, Results	Quantitative analysis, sequential multielement analysis possible in separate analytical cycles, with some spectrometers multielement determination possible by new techniques, especially by high-resolution continuum source AAS (*HR-CS AAS*)
Typical Applications	Quantitative analysis of elements in the trace region. Extremely low detection limits by *ET AAS*. Environmental samples and samples from technical materials. Combination with hydride generation, flow injection analysis (FIA) and solid sampling possible. *F AAS* was in the near past certainly the most commonly used method of elemental analysis of liquid samples and is today in many cases alternatively used to ICP OES

Table 4.6 Optical emission spectrometry

Analytical method	Optical emission spectroscopy (OES) (Atomic emission spectroscopy, AES) Flame-OES, ICP OES, arc-OES, spark-OES, glow-discharge-OES (GD-OES)
Measurement principle	Measurement of the optical emission spectra of atoms or ions due to the excitation with flame (*Flame-OES*), inductively coupled plasma (*ICP OES*, sometimes combined with laser ablation (*LA-ICP OES*) or electrothermal vaporisation (*ETV-ICP OES*)) electrical *arc*, *spark* or glow discharge (*GD-OES*). An emission spectrum consists of lines produced by radiative deexcitation from excited levels of atoms or ions. The intensity of the spectral lines corresponds with the concentration of the element in the plasma
Sample volume/mass	*Flame-OES*: 5–10 mL, *ICP OES*: 1–10 mL Arc-, spark- and glow discharge-OES are direct solid sampling methods *Arc-OES*: 1–50 mg, *Spark-OES*: 10–30 mg, *GD-OES*: ≈ 10 mg (often used for surface/layer analysis)
Typical limits of detection	*Flame-OES*: 1–1000 $\mu g\,L^{-1}$, *ICP OES*: 0.1–50 $\mu g\,L^{-1}$ Spark-OES, GD-OES: 1–100 $mg\,kg^{-1}$ *Arc-OES*, *ETV-ICP OES*: 0.01–10 $mg\,kg^{-1}$
Output, results	Qualitative and quantitative analysis. Determination of traces up to main constituents (depending on the special method). Sequential and simultaneous multielement analysis possible
Typical applications	Qualitative and quantitative elemental analysis of metals and alloys (especially by solid sampling spark-OES), technical materials, environmental, geological and biological samples. ICP OES is the mainly used method for multielement determination in liquid samples

prepared from primary reference materials (elements, compounds and solutions) and the results are directly traceable to the SI provided the purity and stoichiometry of the primary reference materials are assured. With this technique a very high accuracy of the results can be achieved if a multistage process of preparing calibration samples similar to the analyzed sample (this technique of calibration is called *reconstitution analysis*) is carried out [4.130]. Results achieved this way are of high value in metrological interlaboratory comparisons as well as for certification of reference materials.

Total reflection XRF is a very special ultra-trace micro-method for the analysis of objects or layers of very low thickness. The calibration can often carried out using residua of solutions. The widespreading of the method is not high.

Some relevant information concerning the x-ray fluorescence spectrometry is summarized in Table 4.7.

4.3.4 Neutron Activation Analysis (NAA) and Photon Activation Analysis (PAA)

Both methods are characterized by low blank values, because all kinds of chemical handling (such as sample dissolution or surface cleaning) can be done after the step of irradiation, and only the radioactive daughter products of the elements to be determined deliver the measured characteristic signals.

Table 4.7 X-ray fluorescence spectrometry

Analytical method	X-ray fluorescence spectrometry XRF Total reflection x-ray fluorescence spectrometry TXRF
Measurement principle	The solid (or liquid) sample is irradiated with x-rays or a particle beam. Primary radiation is absorbed ejecting inner electrons. Relaxation processes fill the holes and lead to emission of characteristic fluorescence x-rays. The energies (or wavelengths) of these characteristic x-rays are different for each element. Basis for quantitative analysis is the proportionality of the intensity of characteristic x-rays of a certain element and its concentration, but this relation is strongly influenced by the other constituents of the sample *TXRF* is based on the effect of total reflection to achieve extremely high sensitivity
Sample volume/mass	*XRF*: Direct analysis of polished disks of metals or other solid materials or pellets of pressed powders or powders filled into sample cups, thin films, liquids in special sample cells, particulate material on a filter. Typically samples must be flat having surface diameters larger than the diameter of the primary exciting radiation beam. Especially relevant in metrological exercises is the use of fused borate samples in combination with the so-called *reconstitution technique* for calibration *TXRF*: direct analysis of thin (thickness $< 10\,\mu m$) micro-samples or of thin deposits or layers
Typical limits of detection	*XRF*: Strongly dependent from element and matrix as well as from spectrometer (wave length dispersive or energy dispersive). $1-100\,mg\,kg^{-1}$ for elements with medium atomic number, up to some percent for the lightest measurable elements (B, Be) *TXRF*: Down to $\mu g\,L^{-1}$ and below for dried droplets of aqueous solutions
Output, results	Qualitative and quantitative analysis. Semi-quantitative results are easily obtained using special computer programs of the instrument; truly quantitative results require careful sample preparation and calibration of the instrument. Advantages of *XRF* are high precision and wide dynamic range up to high contents
Typical applications	*XRF*: Qualitative and quantitative analysis of metals, alloys, cement, glasses, slag, rocks, inorganic air pollutions, minerals, sediments, freeze-dried biological materials *TXRF*: trace analysis in all kinds of thin micro-samples and residues of solutions for ultra-trace determination

Table 4.8 Neutron and photon activation analysis

Analytical method	Activation analysis NAA, PAA
Measurement principle	Activation analysis is based on the measurement of the radioactivity of indicator radionuclides formed from stable isotopes of the analyte elements by nuclear reactions induced during irradiation of the samples with suitable particles (neutrons: neutron activation analysis *NAA*, high energy photons: photon activation analysis *PAA*)
Sample volume/mass	Solid or (dried) liquid samples: ≈ 50 mg to ≈ 2 g, (in some special systems up to 1 kg)
Typical limits of detection	Trace and ultratrace region, strongly depending on element, sample composition, sub-sample mass and parameters of irradiation. Absolute detection limits: *NAA* 1 pg–10 µg; for most elements: 100 pg–10 ng *PAA* for several nonmetals: 0.1–0.5 µg
Output, results	Qualitative and quantitative analysis. Easy calibration procedure (low matrix influences), high sensitivity and freedom of reagent blanks make in principle the *NAA* (and *PAA*) to important methods with partially very low limits of detection and high accuracy. Homogeneity of elemental distribution can be checked by using small sub-samples. They are important complements to other methods, e.g. to check losses or contamination from wet-chemical sample preparation of the other methods *PAA* is an important complement to NAA, especially in view of determination of nonmetals
Typical applications	*NAA*: Simultaneous trace and ultatrace multielement determination. Nondestructive analysis for ≈ 70 elements (typically up to about 40 elements in one analysis cycle). In principle accurate determination of higher element contents is possible *PAA*: Important especially for determination of nonmetallic analytes. Sample surface can be cleaned after sample irradiation, therefore e.g. very low oxygen blanks are possible for determination of real oxygen bulk content in pure materials

Neutron activation analysis (NAA) normally needs a special nuclear reactor as neutron source. Primarily therefore, the method is not widespread. In principal, up to 70 elements can be determined, however with strongly differing limits of detection. Enormous advantages of the method are the independence of the results from chemical state of analytes and the fact that other matrix effects are marginal mainly according to the high penetration potential of incoming and outgoing radiation. This causes the high metrological value of the method as one being to calibrate easily by pure substances or dried solutions even when the method is used in direct solid sampling mode in form of instrumental NAA (INAA). INAA is the (quasi) nondestructive direct sampling mode of NAA, in opposite to the destructive mode, mostly used as radiochemical NAA (RNAA).

The photon activation analysis (PAA) is complementary to NAA, especially concerning its high sensitivity for light elements, such as nonmetals as C, N, O or F. The method needs a sophisticated device for producing appropriate high energy gamma rays as well as for sample handling and measurement of characteristic signals. Therefore PAA is not a widespread method, even though this method can be of very high analytical importance when low contents of non-metals are to measure accurately at high metrological level.

Some relevant information concerning NAA and PAA is summarized in Table 4.8.

4.4 Compound and Molecular Specific Analysis: Short Surveys of Analytical Methods

Molecular systems can be identified by their characteristic molecular spectra, obtained in the absorption or emission mode from samples in the gaseous, liquid or solid state. Upon interaction with the appropriate

Table 4.9 Basic features of instrumental analytical methods: optical spectroscopy

Analytical method	Measurement principle	Specimen type	Sample amount	Output results	Applications
UV/Vis spectroscopy	Measurement of absorption of emission in the UV/Vis region due to changes of electronic transitions in π-system	Organic compounds, inorganic complexes, ions of transition elements	Solution in transmission, powder in reflexion	Relations between structure and color Photochemical processes	Qualitative and quantitative analysis of aromatic and olefinic compounds Detector for chromatographic methods
Fluorescence spectroscopy	Measurement of fluorescence emission in relation to the wavelength of the excited radiation	Fluorescent organic compounds, dyes, inorganic complex compounds	Solid, liquid or in solution, $\approx 50\,\mu L$	Structure/fluorescencerelations; most sensitive opto-analytical method	Qualitative/quantitative analysis of aromatic compounds with low-energy π–π^* transitions (conjugated chromophors)

Table 4.10 Basic features of instrumental analytical methods: NMR spectroscopy

Analytical method	Measurement principle	Specimen type	Sample amount	Output results	Applications
Nuclear magnetic resonance spectroscopy	Determination of chemical shift and coupling constants due to magnetic field excitation and analysis of RF-emission	Inorganic, organic and organometallic compounds: gaseous, liquid, in solution, solid	Samples in all aggregation states $\approx 100\,\mu g$	Contribution to the molecular structure: bond length, bond angle, interactions about several bonds	Identification of substances in combination with other techniques (MS, IR- and Raman-spectroscopy, chromatography)

Table 4.11 Basic features of instrumental analytical methods: mass spectroscopy

Analytical method	Measurement principle	Specimen type	Sample amount	Output results	Applications
Analytical mass spectroscopy	Generation of gaseous ions from analyte molecules, subsequent separation of these ions according to their mass-to-charge (m/z) ratio, and detection of these ions. Mass spectrum is a plot of the (relatice) abundance of the ions produced as a function of the m/z ratio.	Organic and organometallic compounds: quantitative mixture analysis	Samples in all aggregation states; MS is the most sensitive spectroscopic technique for molecular analysis	Determination of the molecular mass and elemental composition; Contribution to the structure elucidation in combination with NMR, IR- and Raman-spectroscopy	Structure elucidation of unknown compounds; MS as reference method and in the quantitation of drugs; Detector for gas (GC) and liquid chromatographic (LC) methods

Table 4.12 Basic features of instrumental analytical methods. by the methods Infrared, Raman, EPR, Mössbauer Spectroscopy

Analytical method	Measurement principle	Specimen type	Sample amount	Output results	Applications
Infrared spectroscopy	Measurement of absorption (extinction) of radiation in the infrared region due to the modulation of molecular dipolmoment	Inorganic, organic and organometallic compounds: adsorbed molecules	Samples in all aggregation states; In solution, embedded or in matrix isolation, $\approx 100\,\mu$mol	Contribution to the molecular structure: bond length, bond angle, force constants; Identification of characteristic groups and compounds by means of data bases	Identification of substances in combination with other techniques (Raman, NMR, MS, and chromatography); quantitative mixture analysis; Surface analysis of adsorbed molecules; Detector for chromatographic methods
Raman spectroscopy	Measurement of Raman scattering (in the UV-Vis-NIR region) due to the modulation of molecular polarizability	Inorganic, organic and organometallic compounds, surfaces and coatings	Samples in all aggregation states, in solution and in matrix isolation: $\approx 50\,\mu$L, $\approx 1\,\mu$g	Contribution to the molecular structure; Identification of characteristic groups and compounds in combination with IR-data	Identification of substances; Surface and phase analysis; Detector for thin layer chromatographic methods
Electron paramagnetic resonance spectroscopy	Selective absorption of electromagnetic microwaves due to reorientation of magnetic moments of single electrons in a magnetic field	Organic radicals, reactive intermediates Internal defects in solids in biomatrices	In-situ investigation of organic radicals, oriented paramagnetic single crystals, crystal powders	In-situ detection of organic radicals and their reaction kinetics (time-resolved EPR) Paramagnetic centers of crystals	Studies of photochemical and photophysical processes (requirement: 10^{11} single electrons); Semiconductor studies, trace detection of 3-D-elements in biomaterials
Mößbauer spectroscopy	Measurement of the isomeric shift, line width and line intensity due to the recoil free gamma quantum absorption	Inorganic compounds and phases, organometallic compounds	Samples in the solid state, or as freezing solutions, several mg	Determination of the oxidation number and the spin state of the Mössbauer nuclei	Phase analysis including amorphous phase on glasses, ceramics and catalysts

type of electromagnetic radiation, characteristic electronic, vibrational and rotational energy term schemes can be induced in the sample. These excited states usually decay to their ground states within 10^{-2} s, either by emitting the previously absorbed radiation in all directions with the same or lower frequency, or by radiationless relaxation, thus providing spectral information for chemical analysis. Basic features of instrumental analytical methods are summarized in the overview Tables 4.9–4.12 (compiled by Peter Reich, BAM, Berlin, 2004).

Further complementary structural information about molecular systems may be obtained by investigating the nuclear magnetic resonance spectroscopy (NMR) of a sample being irradiated with radio frequency in a magnetic field.

Structural information can also be determined by analysing the intensity distribution mass fragments of a sample bombarded with free electrons, photons or ions in the analytical mass spectroscopy (MS).

Additional information on the near neighbour order in the solid state are provided in particular by the methods infrared (IR) and Raman spectroscopy, EPR and Mössbauer spectroscopy. These techniques provide images of the interactions mentioned above and contain analytical information about the sample.

4.5 National Primary Standards – An Example to Establish Metrological Traceability in Elemental Analysis

Chemical measurements in elemental analysis are measurements of contents (e.g. mass fractions) of analytes in the sample to be analyzed, at which the *chemical identity of the analyte* has to be defined as the element to be measured in the sample. For this purpose, a *metrological traceability system to the SI unit (mol or kg of the chemical element to be measured) for measurement results of inorganic chemical analysis* was set up in Germany in cooperation between the National Metrology and Materials Research Institutes PTB and BAM [4.131, 132]. Currently, the system comprises national primary elemental standards for Cu, Fe, Bi, Ga, Si, Na, K, Sn, W, and Pb and the certification of other elements is in preparation. In this system, core components are

- pure substances (Primary National Standards for Inorganic Chemical Analysis) characterised at the highest metrological level [4.133],
- primary solutions prepared from these pure substances, and
- secondary solutions deduced from the primary solutions intended for transfer to producers of commercial calibration stock solutions and for technical applications.

For certifying a material of a Primary National Standard representing one chemical element in the *System of National Standards for Inorganic Chemical Analysis* all impurities in the material, i. e. all relevant trace elements of the Periodic Table have to be metrologically considered and their mass fractions have to be measured by appropriate analytical methods and then subtracted from 100% mass fraction (= the ideal mass fraction of the investigated element) to establish the real mass fraction of the main component with an uncertainty $< 0.01\%$. This upper limit of the aspired uncertainty is one order of magnitude lower than the lowest uncertainties achieved with direct measurements of the mass fraction of the main component using best metrological methods of elemental analysis, such as IDMS. Even for IDMS measurements the National Standards of Elemental Analysis are intended to be used in the future as instruments of metrological traceability, namely by using them as *natural backspikes for IDMS* of known mass fraction of the main component and therefore as a trustable basis to determine the purity of isotopically enriched spike materials. To determine all trace elements in the pure materials different methods of elemental analysis have to be applied. About 70 metallic impurities can be determined using inductively coupled plasma with high-resolution mass spectrometry (ICP-HRMS). For supplementation and validation, inductively coupled optical emission spectroscopy (ICP OES) and atomic absorption spectrometry (AAS) are used. Classical spectrophotometry is applied for the determination of phosphorous, sulphur and fluorine. Carrier gas hot extraction (CGHE) is used to determine oxygen and nitrogen and combustion analysis is used for carbon and sulphur. In addition to C, O and N, chlorine, bromine and iodine are determined using photon activation analysis (PAA). Hydrogen is measured using nuclear reaction analysis (RNA). For comparison and if possible, also direct methods, typically electrogravimetry (e.g. for copper) or coulometry, are applied in order

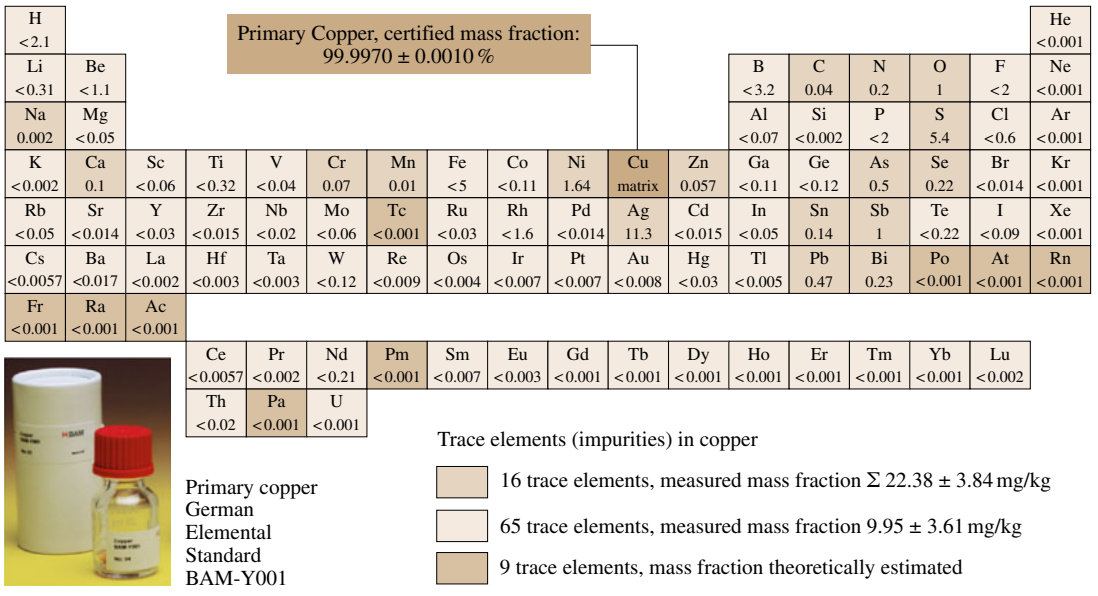

Fig. 4.19 Metrology in elemental analysis. The example of copper as primary standard; mass fractions of trace elements in mg/kg. The expanded uncertainty of matrix element copper has a coverage factor of $k = 2$

to determine the mass fraction of the matrix directly, but of course, with a higher uncertainty.

For those of the high-purity materials, which are most convenient to handle not as pure elements but in the form of salts (alkali metals and alkaline earth metals), additionally to the measurements of the metallic and nonmetallic impurities, the anionic impurities are determined, too. These measurement results are consistent with those of the other element-specific methods for I, Br, S, P or N. For the radioactive elements Tc, Pm, Po, At, Rn, Fr, Ra, Ac and Pa upper limits are estimated from theoretical considerations. For noble gases either measurements are carried out by static mass spectroscopy or limits are estimated from theoretical considerations. All uncertainties are calculated according to the *ISO Guide to the Expression of Uncertainty in Measurement (GUM)*.

An example of this metrology-based system for elemental materials characterisation is given in Fig. 4.19, showing the certified mass fraction data of *primary copper* together with the data of all measured trace elements of the Periodic Table of Chemical Elements. The certified mass fraction of Primary Copper results from subtracting the mass fractions of all impurities from 100%. At this, for each of the 65 values measured below limit of determination or of the 9 values estimated from theoretical considerations below a limit value, half of the limit value (having an assumed uncertainty of (± half of the limit value)) was subtracted from 100%.

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