– the crystal density which only includes the solid fraction of the material; the crystal density is also called true density;
– the particle density which also includes the volume due to intraparticulate pores,
– the bulk density which further includes the interparticulate void volume formed in the powder bed; the bulk density is also called apparent density.

CRystal DENSITY
The crystal density of a substance is the average mass per unit volume, exclusive of all voids that are not a fundamental part of the molecular packing arrangement. It is an intrinsic property of the substance, and hence should be independent of the method of determination. The crystal density can be determined either by calculation or by simple measurement.

A. The calculated crystal density is obtained using crystallographic data (size and composition of the unit cell) of a perfect crystal, from for example X-ray diffraction data, and the molecular mass of the substance.
B. The measured crystal density is the mass to volume ratio after measuring the monocrystal mass and volume.

PARTICLE DENSITY
The particle density takes into account both the crystal density and the intraparticulate porosity (sealed and/or open pores). Thus, particle density depends on the value of the volume determined which in turn depends on the method of measurement. The particle density can be determined using one of the two following methods.

A. The pycnometric density is determined by measuring the volume occupied by a known mass of powder which is equivalent to the volume of gas displaced by the powder using a gas displacement pycnometer (2.9.23). In pycnometric density measurements, the volume determined includes the volume occupied by open pores; however, it excludes the volume occupied by sealed pores or pores inaccessible to the gas. Due to the high diffusivity of helium, which is the preferred choice of gas, most open pores are accessible to the gas. Therefore, the pycnometric density of a finely milled powder is generally not very different from the crystal density.
B. The mercury porosimeter density is also called granular density. With this method the volume determined also excludes contributions from sealed pores; however, it includes the volume only from open pores larger than some size limit. This pore size limit or minimal access diameter depends on the maximal mercury intrusion pressure applied during the measurement and under normal operating pressures the mercury does not penetrate the finest pores accessible to helium. Various granular densities can be obtained from one sample since, for each applied mercury intrusion pressure, a density can be determined that corresponds to the pore size limit at that pressure.

BULK AND TAPPED DENSITY
The bulk density of a powder includes the contribution of interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the space arrangement of particles in the powder bed.
The bulk density of a powder is often very difficult to measure since the slightest disturbance of the bed may result in a new density. Thus, it is essential in reporting bulk density to specify how the determination was made.
A. The bulk density is determined by measuring the volume of a known mass of powder, that has been passed through a screen, into a graduated cylinder (2.9.15).
B. The tapped density is achieved by mechanically tapping a measuring cylinder containing a powder sample. After observing the initial volume, the cylinder is mechanically tapped, and volume readings are taken until little further volume change is observed (2.9.15).

2.2.43. Mass spectrometry

Mass spectrometry is based on the direct measurement of the ratio of the mass to the number of positive or negative elementary charges of ions (m/z) in the gas phase obtained from the substance to be analysed. This ratio is expressed in atomic mass units (1 a.m.u. = one twelfth the mass of 12C) or in daltons (1 Da = the mass of the hydrogen atom).
The ions, produced in the ion source of the apparatus, are accelerated and then separated by the analyser before reaching the detector. All of these operations take place in a chamber where a pumping system maintains a vacuum of $10^{-3}$ to $10^{-6}$ Pa.
The resulting spectrum shows the relative abundance of the various ionic species present as a function of m/z.
The signal corresponding to an ion will be represented by several peaks corresponding to the statistical distribution of the various isotopes of that ion. This pattern is called the isotopic profile and (at least for small molecules) the peak representing the most abundant isotopes for each atom is called the monoisotopic peak.

Information obtained in mass spectrometry is essentially qualitative (determination of the molecular mass, information on the structure from the fragments observed) or quantitative (using internal or external standards) with limits of detection ranging from the picomole to the femtomole.

INTRODUCTION OF THE SAMPLE
The very first step of an analysis is the introduction of the sample into the apparatus without overly disturbing the vacuum. In a common method, called direct liquid introduction, the sample is placed on the end of a cylindrical rod (in a quartz crucible, on a filament or on a metal surface). This rod is introduced into the spectrometer after passing through a vacuum lock where a primary intermediate vacuum is maintained between atmospheric pressure and the secondary vacuum of the apparatus.
Other introduction systems allow the components of a mixture to be analysed as they are separated by an appropriate apparatus connected to the mass spectrometer.

Gas chromatography/mass spectrometry. The use of suitable columns (capillary or semi-capillary) allows the end of the column to be introduced directly into the source of the apparatus without using a separator.

Liquid chromatography/mass spectrometry. This combination is particularly useful for the analysis of polar compounds, which are insufficiently volatile or too heat-labile to be analysed by gas chromatography coupled with mass spectrometry. This method is complicated by the difficulty of obtaining ions in the gas phase from a liquid phase, which requires very special interfaces such as:
– direct liquid introduction: the mobile phase is nebulised, and the solvent is evaporated in front of the ion source of the apparatus,
– particle-beam interface: the mobile phase, which may flow at a rate of up to 0.6 ml/min, is nebulised in a desolvation chamber such that only the analytes, in neutral form, reach the ion source of the apparatus; this technique is used for compounds of relatively low polarity with molecular masses of less than 1000 Da.

– moving-belt interface: the mobile phase, which may flow at a rate of up to 1 ml/min, is applied to the surface of a moving belt; after the solvent evaporates, the components to be analysed are successively carried to the ion source of the apparatus where they are ionised; this technique is rather poorly suited to very polar or heat-labile compounds.

Other types of coupling (electrospray, thermospray, atmospheric-pressure chemical ionisation) are considered to be ionisation techniques in their own right and are described in the section on modes of ionisation.

Supercritical fluid chromatography/mass spectrometry. The mobile phase, usually consisting of supercritical carbon dioxide enters the gas state after passing a heated restrictor between the column and the ion source.

Capillary electrophoresis/mass spectrometry. The eluent is introduced into the ion source, in some cases after adding another solvent so that flow rates of the order of a few microlitres per minute can be attained. This technique is limited by the small quantities of sample introduced and the need to use volatile buffers.

MODES OF IONISATION

Electron impact. The sample, in the gas state, is ionised by a beam of electrons whose energy (usually 70 eV) is greater than the ionisation energy of the sample. In addition to the molecular ion M+, fragments characteristic of the molecular structure are observed. This technique is limited mainly by the need to vapourise the sample. This makes it unsuited to polar, heat-labile or high molecular mass compounds. Electron impact is compatible with the coupling of gas chromatography to mass spectrometry and sometimes with the use of liquid chromatography.

Chemical ionisation. This type of ionisation involves a reagent gas such as methane, ammonia, nitrogen oxide, nitrogen dioxide or oxygen. The spectrum is characterised by ions of the (M + H)+ type or adduct ions formed from the matrix or the gas used. Fewer fragments are produced than with electron impact. A variant of this technique is used when the substance is heat-labile: the sample, applied to a filament, is very rapidly vaporised by the Joule-Thomson effect (desorption chemical ionisation).

Fast-atom bombardment (FAB) or fast-ion bombardment ionisation (liquid secondary-ion mass spectrometry LSIMS). The sample, dissolved in a viscous matrix such as glycerol, is applied to a metal surface and ionised by a beam of neutral atoms such as argon or xenon or high-kinetic-energy caesium ions. Ions of the (M + H)+ or (M – H)+ types or adduct ions formed from the matrix or the sample are produced. This type of ionisation, well suited to polar and heat-labile compounds, allows molecular masses of up to 10 000 Da to be obtained. The technique can be combined with liquid chromatography by adding 1 per cent to 2 per cent of glycerol to the mobile phase; however, the flow rates must be very low (a few microlitres per minute). These ionisation techniques also allow thin-layer chromatography plates to be analysed by applying a thin layer of matrix to the surface of these plates.

Field desorption and field ionisation. The sample is vapourised near a tungsten filament covered with microneedles (field ionisation) or applied to this filament (field desorption). A voltage of about 10 kV, applied between this filament and a counter-electrode, ionises the sample. These two techniques mainly produce molecular ions M+ and (M + H)+ ions and are used for low polarity and/or heat-labile compounds.

Matrix-assisted laser desorption ionisation (MALDI). The sample, in a suitable matrix and deposited on a metal support, is ionised by a pulsed laser beam whose wavelength may range from UV to IR (impulses lasting from a picosecond to a few nanoseconds). This mode of ionisation plays an essential role in the analysis of very high molecular mass compounds (more than 100 000 Da) but is limited to time-of-flight analysers (see below).

Electrospray. This mode of ionisation is carried out at atmospheric pressure. The samples, in solution, are introduced into the source through a capillary tube, the end of which has a potential of the order of 5 kV. A gas can be used to facilitate nebulisation. Desolvation of the resulting microdroplets produces singly or multiply charged ions in the gas phase. The flow rates vary from a few microlitres per minute to 1 ml/min. This technique is suited to polar compounds and to the investigation of biomolecules with molecular masses of up to 100 000 Da. It can be coupled to liquid chromatography or capillary electrophoresis.

Atmospheric-pressure chemical ionisation (APCI). Ionisation is carried out at atmospheric pressure by the action of an electrode maintained at a potential of several kilovolts and placed in the path of the mobile phase, which is nebulised both by thermal effects and by the use of a stream of nitrogen. The resulting ions carry a single charge and are of the (M + H)+ type in the positive mode and of the (M – H)+ type in the negative mode. The high flow rates that can be used with this mode of ionisation (up to 2 ml/min) make this an ideal technique for coupling to liquid chromatography.

Thermospray. The sample, in the mobile phase consisting of water and organic modifiers and containing a volatile electrolyte (generally ammonium acetate) is introduced in nebulised form after having passed through a metal capillary tube at controlled temperature. Acceptable flow rates are of the order of 1 ml/min to 2 ml/min. The ions of the electrolyte ionise the compounds to be analysed. This ionisation process may be replaced or enhanced by an electrical discharge of about 800 volts, notably when the solvents are entirely organic. This technique is compatible with the use of liquid chromatography coupled with mass spectrometry.

ANALYSERS

Differences in the performance of analysers depend mainly on two parameters:

– the range over which m/z ratios can be measured, ie, the mass range,

– their resolving power characterised by the ability to separate two ions of equal intensity with m/z ratios differing by ΔM, and whose overlap is expressed as a given percentage of valley definition; for example, a resolving power (M/ΔM) of 1000 with 10 per cent valley definition allows the separation of m/z ratios of 1000 and 1001 with the intensity returning to 10 per cent above baseline. However, the resolving power may in some cases (time-of-flight analysers, quadrupoles, ion-trap analysers) be defined as the ratio between the molecular mass and peak width at half height (50 per cent valley definition).

Magnetic and electrostatic analysers. The ions produced in the ion source are accelerated by a voltage V, and focused towards a magnetic analyser (magnetic field B) or an
2.2.43. Mass spectrometry

EUROPEAN PHARMACOPOEIA 6.0

2. Methods of analysis

2.6. Mass spectrometry

Ion-trap analyser. The principle is the same as for a quadrupole, this time with the electric fields in three dimensions. This type of analyser allows production spectra over several generations (MS^n) to be obtained.

Ion-cyclotron resonance analysers. Ions produced in a cell and subjected to a uniform, intense magnetic field move in circular orbits at frequencies which can be directly correlated to their m/z ratio by applying a Fourier transform algorithm. This phenomenon is called ion-cyclotron resonance. Analysers of this type consist of superconducting magnets and are capable of very high resolving power (up to 10 000 000 and more) as well as MS^n spectra. However, very low pressures are required (of the order of 10^-7 Pa).

Time-of-flight analysers. The ions produced at the ion source are accelerated at a voltage V of 10 kV to 20 kV. They pass through the analyser, consisting of a field-free tube, 25 cm to 1.5 m long, generally called a flight tube. The time (t) for an ion to travel to the detector is proportional to the square root of the m/z ratio. Theoretically the mass range of such an analyser is infinite. In practice, it is limited by the ionisation or desorption method. Time-of-flight analysers are mainly used for high molecular mass compounds (up to several hundred thousand daltons). This technique is very sensitive (a few picomoles of product are sufficient). The accuracy of the measurements and the resolving power of such instruments may be improved considerably by using an electrostatic mirror (reflectron).

SIGNAL ACQUISITION

There are essentially three possible modes.

Complete spectrum mode. The entire signal obtained over a chosen mass range is recorded. The spectrum represents the relative intensity of the different ionic species present as a function of m/z. The results are essentially qualitative. The use of spectral reference libraries for more rapid identification is possible.

Fragmentometric mode (Selected-ion monitoring). The acquired signal is limited to one (single-ion monitoring (SIM)) or several (multiple-ion monitoring (MIM)) ions characteristic of the substance to be analysed. The limit of detection can be considerably reduced in this mode. Quantitative or semiquantitative tests can be carried out using external or internal standards (for example, deuterated standards). Such tests cannot be carried out with time-of-flight analysers.

Fragmentometric double mass spectrometry mode (multiple reaction monitoring (MRM)). The unimolecular or bimolecular decomposition of a chosen precursor ion characteristic of the substance to be analysed is followed specifically. The selectivity and the highly specific nature of this mode of acquisition provide excellent sensitivity levels and make it the most appropriate for quantitative studies using suitable internal standards (for example, deuterated standards). Such analysis can be performed only on apparatus fitted with three quadrupoles in series, ion-trap analysers or cyclotron-resonance analysers.

CALIBRATION

Calibration allows the corresponding m/z value to be attributed to the detected signal. As a general rule, this is done using a reference substance. This calibration may be external (acquisition file separate from the analysis) or internal (the reference substance(s) are mixed with the substance to be examined and appear on the same acquisition file). The number of ions or points required for reliable calibration depends on the type of analyser and on the desired accuracy of the measurement, for example, in

electrostatic analyser (electrostatic field \( E \)), depending on the configuration of the instrument. They follow a trajectory of radius \( r \) according to Laplace’s law:

\[
\frac{m}{z} = \frac{B^2 e^2}{2V}
\]

Two types of scans can be used to collect and measure the various ions produced by the ion source: a scan of B holding V fixed or a scan of V with constant B. The magnetic analyser is usually followed by an electric sector that acts as a kinetic energy filter and allows the resolving power of the instrument to be increased appreciably. The maximum resolving power of such an instrument (double sector) ranges from 10 000 to 15 000 and in most cases allows the value of \( m/z \) ratios to be calculated accurately enough to determine the elemental composition of the corresponding ions. For monocharged ions, the mass range is from 2000 Da to 15 000 Da. Some ions may decompose spontaneously (metastable transitions) or by colliding with a gas (collision-activated dissociation (CAD)) in field-free regions between the ion source and the detector. Examination of these decompositions is very useful for the determination of the structure as well as the characterisation of a specific compound in a mixture and involves tandem mass spectrometry. There are many such techniques depending on the region where these decompositions occur:

- daughter-ion mode (determination of the decomposition ions of a given parent ion): B/\( E \) = constant, MIKES (Mass-analysed Ion Kinetic Energy Spectroscopy),
- parent-ion mode (determination of all ions which by decomposition give an ion with a specific \( m/z \) ratio): \( B/\sqrt{E} \) = constant,
- neutral-loss mode (determination of all the ions that lose the same fragment):
  \[
  \frac{m}{z} = \frac{B}{E}(1 - E/\sqrt{E})^{1/2} = \text{constant}, \]
  where \( E \) is the basic voltage of the electric sector.

Quadrupoles. The analyser consists of four parallel metal rods, which are cylindrical or hyperbolic in cross-section. They are arranged symmetrically with respect to the trajectory of the ions; the pairs diagonally opposed about the axis of symmetry of rods are connected electrically. The potentials to the two pairs of rods are opposed. They are the resultant of a constant component and an alternating component. The ions produced at the ion source are transmitted and separated by varying the voltages applied to the rods so that the ratio of continuous voltage to alternating voltage remains constant. The quadrupoles usually have a mass range of 1 a.m.u. to 2000 a.m.u., but some may range up to 4000 a.m.u. Although they have a lower resolving power than magnetic sector analysers, they nevertheless allow the monoisotopic profile of single charged ions to be obtained for the entire mass range. It is possible to obtain spectra using three quadrupoles arranged in series, \( Q_1 \), \( Q_2 \), \( Q_3 \) (\( Q_3 \) serves as a collision cell and is not really an analyser; the most commonly used collision gas is argon).

The most common types of scans are the following:

- daughter-ion mode: \( Q_3 \) selects an \( m/z \) ion whose fragments obtained by collision in \( Q_2 \) are analysed by \( Q_3 \),
- parent-ion mode: \( Q_2 \) filters only a specific \( m/z \) ratio, while \( Q_3 \) scans a given mass range. Only the ions decomposing to give the ion selected by \( Q_2 \) are detected,
- neutral loss mode: \( Q_1 \) and \( Q_3 \) scan a certain mass range but at an offset corresponding to the loss of a fragment characteristic of a product or family of compounds.

It is also possible to obtain spectra by combining quadrupole analysers with magnetic or electrostatic sector instruments; such instruments are called hybrid mass spectrometers.
the case of a magnetic analyser where the m/z ratio varies exponentially with the value of the magnetic field, there should be as many points as possible.

**SIGNAL DETECTION AND DATA PROCESSING**

Ions separated by an analyser are converted into electric signals by a detection system such as a photomultiplier or an electron multiplier. These signals are amplified before being re-converted into digital signals for data processing, allowing various functions such as calibration, reconstruction of spectra, automatic quantification, archiving, creation or use of libraries of mass spectra. The various physical parameters required for the functioning of the apparatus as a whole are controlled by computer.

01/2008:20244

### 2.2.44. TOTAL ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE

Total organic carbon (TOC) determination is an indirect measure of organic substances present in water for pharmaceutical use. TOC determination can also be used to monitor the performance of various operations in the preparation of medicines.

A variety of acceptable methods is available for determining TOC. Rather than prescribing a given method to be used, this general chapter describes the procedures used to qualify the chosen method and the interpretation of results in limit tests. A standard solution is analysed at suitable intervals, depending on the frequency of measurements; the solution is prepared with a substance that is expected to be easily oxidisable (for example, sucrose) at a concentration adjusted to give an instrument response corresponding to the TOC limit to be measured. The suitability of the system is determined by analysis of a solution prepared with a substance expected to be oxidisable with difficulty (for example, 1,4-benzoquinone).

The various types of apparatus used to measure TOC in water for pharmaceutical use have in common the objective of completely oxidising the organic molecules in the sample water to produce carbon dioxide followed by measurement of the amount of carbon dioxide produced, the result being used to calculate the concentration of carbon in the water.

The apparatus used must discriminate between organic and inorganic carbon, the latter being present as carbonate. The discrimination may be effected either by measuring the inorganic carbon and subtracting it from the total carbon, or by purging inorganic carbon from the sample before oxidation. Purging may also entrain organic molecules, but such purgeable organic carbon is present in negligible quantities in water for pharmaceutical use.

**Apparatus.** Use a calibrated instrument installed either on-line or off-line. Verify the system suitability at suitable intervals as described below. The apparatus must have a limit of detection specified by the manufacturer of 0.05 mg or less of carbon per litre.

**TOC water.** Use highly purified water complying with the following specifications:
- conductivity: not greater than 1.0 µS cm⁻¹ at 25 °C,
- total organic carbon: not greater than 0.1 mg/l.

Depending on the type of apparatus used, the content of heavy metals and copper may be critical. The manufacturer’s instructions should be followed.

Glassware preparation. Use glassware that has been scrupulously cleaned by a method that will remove organic matter. Use TOC water for the final rinse of glassware.

**Standard solution.** Dissolve sucrose R, dried at 105 °C for 3 h in TOC water to obtain a solution containing 1.19 mg of sucrose per litre (0.50 mg of carbon per litre).

**Test solution.** Using all due care to avoid contamination, collect water to be tested in an airtight container leaving minimal head-space. Examine the water with minimum delay to reduce contamination from the container and its closure.

**System suitability solution.** Dissolve 1,4-benzoquinone R in TOC water to obtain a solution having a concentration of 0.75 mg of 1,4-benzoquinone per litre (0.50 mg of carbon per litre).

**TOC water control.** Use TOC water obtained at the same time as that used to prepare the standard solution and the system suitability solution.

**Control solutions.** In addition to the TOC water control, prepare suitable blank solutions or other solutions needed for establishing the baseline or for calibration adjustments following the manufacturer’s instructions; run the appropriate blanks to zero the instrument.

**System suitability.** Run the following solutions and record the responses: TOC water (r₁); standard solution (r₂); system suitability solution (r₃). Calculate the percentage response efficiency using the expression:

\[
\frac{r₃ - r₁}{r₂ - r₁} \times 100
\]

The system is suitable if the response efficiency is not less than 85 per cent and not more than 115 per cent of the theoretical response.

**Procedure.** Run the test solution and record the response (r₃). The test solution complies with the test if r₃ is not greater than r₁ - r₂.

The method can also be applied using on-line instrumentation that has been adequately calibrated and shown to have acceptable system suitability. The location of instrumentation must be chosen to ensure that the responses are representative of the water used.

01/2008:20245

### 2.2.45. SUPERCRITICAL FLUID CHROMATOGRAPHY

Supercritical fluid chromatography (SFC) is a method of chromatographic separation in which the mobile phase is a fluid in a supercritical or subcritical state. The stationary phase, contained in a column, consists of either finely divided solid particles, such as a silica or porous graphite, a chemically modified stationary phase, as used in liquid chromatography, or, for capillary columns, a cross-linked liquid film evenly coated on the walls of the column.

SFC is based on mechanisms of adsorption or mass distribution.

**APPARATUS**

The apparatus usually consists of a cooled pumping system, an injector, a chromatographic column, contained in an oven, a detector, a pressure regulator and a data acquisition device (or an integrator or a chart recorder).

**Pumping system**

Pumping systems are required to deliver the mobile phase at a constant flow rate. Pressure fluctuations are to be