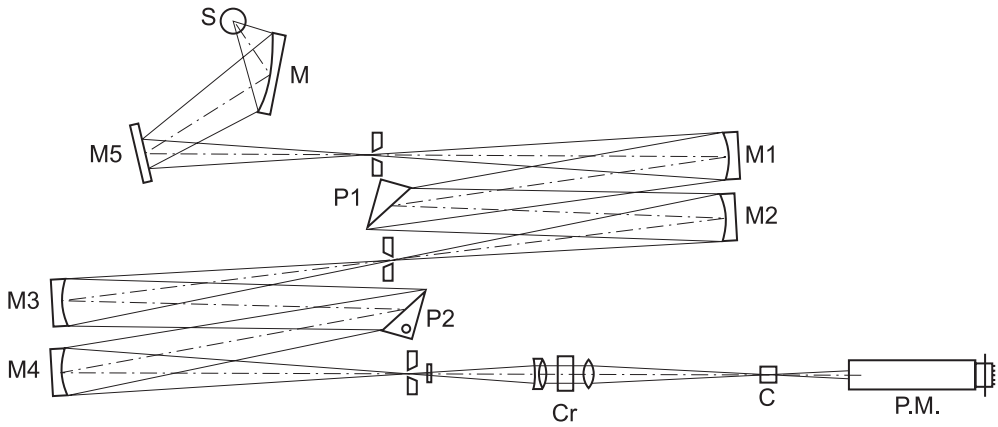

Figure 2.2.41.-1. — *Optical scheme of a dichrograph*

M = relative molecular mass of the substance to be examined,

c = concentration of the solution to be examined in g/ml,

l = optical path of the cell in centimetres.

Molar ellipticity is also related to molar circular dichroism by the following equation:

$$[\Theta] = 2.303\Delta\varepsilon\frac{4500}{\pi} \approx 3300\Delta\varepsilon$$

Molar ellipticity is often used in the analysis of proteins and nucleic acids. In this case, molar concentration is expressed in terms of monomeric residue, calculated using the expression:

$$\frac{\text{molecular mass}}{\text{number of amino acids}}$$

The mean relative molecular mass of the monomeric residue is 100 to 120 (generally 115) for proteins and about 330 for nucleic acids (as the sodium salt).

Apparatus. The light source (S) is a xenon lamp (Figure 2.2.41.-1); the light passes through a double monochromator (M) equipped with quartz prisms (P1, P2).

The linear beam from the first monochromator is split into 2 components polarised at right angles in the second monochromator. The exit slit of the monochromator eliminates the extraordinary beam.

The polarised and monochromatic light passes through a birefringent modulator (Cr): the result is alternating circularly polarised light.

The beam then passes through the sample to be examined (C) and reaches a photomultiplier (PM) followed by an amplifier circuit which produces 2 electrical signals: one is a direct current V_c and the other is an alternating current at the modulation frequency V_{ac} characteristic of the sample to be examined. The phase gives the sign of the circular dichroism. The ratio V_{ac}/V_c is proportional to the differential absorption ΔA which created the signal. The region of wavelengths normally covered by a dichrograph is 170 nm to 800 nm.

Calibration of the apparatus

Accuracy of absorbance scale. Dissolve 10.0 mg of *isoandrosterone R* in *dioxan R* and dilute to 10.0 ml with the same solvent. Record the circular dichroism spectrum of the solution between 280 nm and 360 nm. Measured at the maximum at 304 nm, $\Delta\varepsilon$ is + 3.3.

The solution of *(1S)-(+)-10-camphorsulphonic acid R* may also be used.

Linearity of modulation. Dissolve 10.0 mg of *(1S)-(+)-10-camphorsulphonic acid R* in *water R* and dilute to 10.0 ml with the same solvent. Determine the exact concentration of camphorsulphonic acid in the solution by ultraviolet spectrophotometry (*2.2.25*), taking the specific absorbance to be 1.49 at 285 nm.

Record the circular dichroism spectrum between 185 nm and 340 nm. Measured at the maximum at 290.5 nm, $\Delta\varepsilon$ is + 2.2 to + 2.5. Measured at the maximum at 192.5 nm, $\Delta\varepsilon$ is −4.3 to −5.

(1S)-(+)- or antipodal *(1R)-(−)-ammonium 10-camphorsulphonate R* can also be used.

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2.2.42. DENSITY OF SOLIDS

The density of solids corresponds to their average mass per unit volume and typically is expressed in grams per cubic centimetre (g/cm^3) although the International Unit is the kilogram per cubic meter (1 g/cm^3 = 1000 kg/m^3).

Unlike gases and liquids whose density depends only on temperature and pressure, the density of a solid particle also depends on its molecular assembly and therefore varies with the crystal structure and degree of crystallinity.

When a solid particle is amorphous or partially amorphous, its density may further depend upon the history of preparation and treatment.

Therefore, unlike fluids, the densities of two chemically equivalent solids may be different, and this difference reflects a difference in solid-state structure. The density of constituent particles is an important physical characteristic of pharmaceutical powders.

The density of a solid particle can assume different values depending on the method used to measure the volume of the particle. It is useful to distinguish three levels of expression of density:

- 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*).

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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 ^{12}C) 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.