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Selecting a Particle Sizer for the Pharmaceutical Industry

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1.1 Introduction

1.1.1 Relevance of Particle Size in the Pharmaceutical Industry

Knowledge and understanding of particle size data is crucial in a wide range of industries, being vital for the pharmaceutical industry, with applications from drug development to production and quality control. The purpose of particle size analysis is to obtain quantitative data on the mean size, particle size distribution and, sometimes in addition, particle shape of the compounds used in pharmaceutical formulations. It is well known that particle size highly affects not only the final product performance (e.g., dissolution, bioavailability, stability and absorption rates), but also every step of the manufacturing process of both drug substances and excipients (like mixing, flowability, granulation, drying, milling, blending, coating and encapsulation) [1–8]. For example, particle size is often directly related to dissolution/solubility characteristics of solid or suspension delivery systems, which have a direct impact on the bioavailability of pharmaceutical products. Dissolution is directly proportional to particle surface area, which in turn is inversely proportional to particle size (i.e., finer particles promote faster drug dissolution). The same applies to the suspensions where precipitation is highly controlled by particle size (in practice, finer particles generally give more stable suspensions), equally affecting viscosity and flow (Stokes' law relates the settling velocity of particles to the square

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of particle diameter). Distribution of sizes is another key characteristic that influences, for instance, handling and processing (powder handling characteristics are profoundly affected by changes in flow properties and tendency to segregate, which are both highly dependent on powder size distribution). Ultimately, particle size also has a critical effect on the content uniformity of solid dosage forms. Size analysis also becomes of significant importance with new drug delivery formats such as liposomes and nanoparticles whose characterization requires sophisticated analytical techniques [9–12].

In brief, particle size simultaneously affects safety, efficacy and quality of the drug, and regulatory agencies are becoming increasingly aware of the importance of particle sizing, requiring developers to have greater control and understanding of this aspect of their drug products [3, 13–15].

1.1.2 Main Goals

This chapter intends to introduce the problem of particle sizing in the domain of the pharmaceutical industry, especially to those who are not very familiar with this topic. It is by no means an exhaustive description of particle sizing methods, but addresses the basic concepts associated with particle sizing, providing a basis to understand the most important details associated with particle sizing data and its interpretation. It was conceived not only to help the reader to select the most suitable techniques for your particle characterization needs, but also to be a valuable tool in daily work situations.

A considerable effort was made to condense in a single chapter topics that range from the interpretation of sizing data to the working principles, applications and limitations of some selected methods, including their selection criteria, subjects that are normally treated in separate publications/chapters. The idea was to provide the essential information to enable the reader to completely follow all the topics covered here. After discussing the reasons why choosing a particle sizer is not an easy task, some basic definitions of particle size, size distribution and their representations will be given in a concise manner, before addressing some of the most relevant parameters to be taken into consideration when selecting a particle sizing method. Finally, the underlying principles of some selected methods will be presented, together with their strengths and weaknesses. Naturally, the number of addressed methods had to be limited. Hence, this discussion will mainly be directed to sizing techniques normally available for *routine analyses* in the *pharmaceutical field*, from *nanoparticles* to some *hundred micrometer particles*. In order to encompass one of each class of particle sizing methods, the following techniques were selected: optical microscopy/image analysis and time-of-flight, representative of the counting techniques; static and dynamic light scattering, widely used ensemble techniques; and the cascade impactor, a separation technique frequently used for aerosol samples (nasal products). As mentioned, the ultimate goal will be to stimulate the reader's curiosity to consult other sources of information to complement this analysis.

1.1.3 Why it is So Difficult to Select a Particle Sizer

The apparent simplicity of particle size analysis is deceptive as particle sizing is a poorly posed problem. As is well known, only objects of simple geometry, namely spheres, can

be unambiguously described by a single linear dimension. Non-spherical particles, as discussed below, are most conveniently described in terms of derived diameters calculated by measuring a size-dependent property of the particle and relating it to a linear dimension. As a result, different sizing methods, based on the measurement of different particle properties, might give different sizing data for the same sample. Moreover, the same measuring technique can also generate different sizing results as a consequence of distinct data processing algorithms used by the equipment manufacturers [2, 3, 16, 17]. Complicating this, a wide range of size distributions normally have to be analysed, being not uncommon that the size range of the particles is too wide to be measured with a single device. Besides, particles, namely pharmaceuticals, include dry powders, suspensions, aerosols, emulsions and nanoparticles, which in turn can be presented as primary (individual) particles, aggregates or agglomerates (in *aggregates* the primary particles are bound strongly by covalent bonds, whereas *agglomerates* are collections of aggregates loosely held together by weak forces). Also, the recent interest in measuring nanoparticles resulted in a burst of new techniques (or new applications of old techniques) for the nanometer range, being that the smaller the particles, the more difficult it is to characterize them. Accordingly, there has never been so much diversity of sizing equipment (hundreds of commercially available instruments), sample and data treatments.

Additionally, it should be pointed out that formal training in the field of particle technology is not often as widespread as in other fields. Further, the technical information available in particle technology, namely particle sizing vocabulary, is unique and complex, and a clear domain of fine particle technology terminology is indispensable for correct data interpretation.

As a final point, it should be highlighted that the determination of particle size distribution seldom is the ultimate objective: indeed, a particle size measurement is often carried out with the aim of relating particle sizing data to a particular property or behavior of the material, and this relationship should be taken into consideration when choosing a sizing instrument. For example, if we are studying the particles of an airborne aerosol and their deposition in the lungs, a sizing method based on the measurement of the aerodynamic diameter would be more appropriate; furthermore, if a drug product is to be administered as a dry powder, a particle characterization technique capable of measuring the sample as a dry powder dispersion should be used.

Sizing equipment is not often restricted to a specific application, being normally used for more general purposes. Nonetheless, it should be borne in mind that no single technique is superior in all applications. All these reasons render the selection of the most appropriate particle sizing method a challenging process.

1.2 Particle Size Distribution

1.2.1 Equivalent Diameter

It is not possible to rationally discuss the size of a particle without considering the three-dimensional characteristics of the particle itself (length, breadth, and height). In fact, only the sphere can be fully described by a single dimension, its radius or diameter. However, most real-world particles are far from round or uniform, and with regard to particle sizing, it is often most convenient to express particle size in terms of derived diameters

such as equivalent spherical diameter (ESD). ESD is defined by ISO 9276-1:1998 [18] as the diameter of a sphere that produces a response by a given particle-sizing method that is equivalent to the response produced by the particle being measured. In many cases the equivalent sphere is the one with the same volume as the particle, the so-called volume-equivalent spherical diameter (a cube of length 1 μm has a volume-equivalent spherical diameter of 1.24 μm). However, the method of measurement and the property of interest of the particle can lead to the use of other diameters, such as, for instance, the surface-equivalent spherical diameter, which is the diameter of a sphere having the same surface area as the particle, or the projected area diameter, most used in image analysis, that is the diameter of a circle having the same area as the projected area of the particle. These and other frequently used particle-equivalent diameters are defined in Table 1.1 [16].

Table 1.1 *Definitions of equivalent spherical diameters (ESDs)*

ESD	Definition
Volume diameter	Diameter of a sphere having the same volume as the particle
Surface diameter	Diameter of a sphere having the same (external) surface area as the particle
Projected area diameter	Diameter of a circle having the same area as the projected area of the particle resting in a stable position
Surface volume diameter (Sauter diameter)	Diameter of a sphere having the same surface area-to-volume ratio as the particle
Sieve diameter	Diameter of a sphere passing through a sieve of defined mesh size (with square or circular apertures)
Stokes diameter	Diameter of a sphere with the same final settling velocity as the particle undergoing laminar flow in a fluid of the same density and viscosity
Hydrodynamic diameter	Diameter of a sphere with the same translational diffusion coefficient as the particle in the same fluid under the same conditions
Mobility diameter	Diameter of a sphere having the same mobility in an electric field as the particle
Fraunhofer diameter	Diameter of a sphere that will scatter light at the same intensity at the same angle as the particle (correspond to the projected area diameter of a particle in random orientation)
Optical diameter	Diameter of a sphere having the same optical cross-section as the particle
Aerodynamic diameter	Diameter of a unit density sphere that would have the identical settling velocity as the particle

Clearly, non-spherical particles can lead to very different equivalent diameters depending on the definition chosen, which in turn is related to the measured particle property and ultimately to the sizing instrument/technique used. The further away from spherical the actual particle shape is, the greater the difference in ESD (for non-spherical compact convex particles, the results will not differ greatly for the various size measurements, but for needles, disks or flakes, with one dimension significantly different from the others, the differences may be quite relevant). Moreover, ESD may not correlate with any single dimension of the particle. On the other hand, identical equivalent diameters may be obtained for different

particle shapes. For that, particle size and particle size distribution results are frequently considered as relative measurements, and comparisons of size results from different instruments should be conducted with extreme caution.

Although the ESD approach is simplistic, it is very convenient and it is employed in almost all particle sizing techniques. However, it is absolutely essential to be clear and consistent as to which ESD is being used.

1.2.2 Reporting Particle Size

This section briefly addresses the representation of size distributions, focusing mainly on the types of curves used to express the distribution and some central tendencies. Nowadays, all particle sizers report the data in graphical form (some of which we can select) indicating some statistical parameters. However, a perfect understanding of the distributions and of the associated statistical parameters is absolutely essential for a correct interpretation of the sizing data [16, 19–22].

Almost all real-world samples exist as a distribution of particle sizes, normally expressed as a function of two coordinates: the size (mostly an ESD) plotted on the *x*-axis, and the amount of each size, plotted on the *y*-axis, as illustrated in Figure 1.1. The size distribution can be represented in the form of either a frequency (differential) distribution curve or a cumulative (normally undersize) distribution curve (typically with a sigmoidal shape), obtained by sequentially adding the percentage frequency values. Both types of plot are useful depending on the information we want from the graphical representation: the frequency distribution presents a clear description of the distribution spread and also shows if the distribution is monomodal or multimodal (i.e., with one or more peaks, respectively) and whether the peak is skewed from the centre; in a cumulative plot, multimodal peaks are not easily observed but the identification of the percentage of particles below a given diameter is much simpler.

However, we need to be aware that, depending on the sizing technique, the amount of each size can be weighted in different ways [23]. The more common weighted

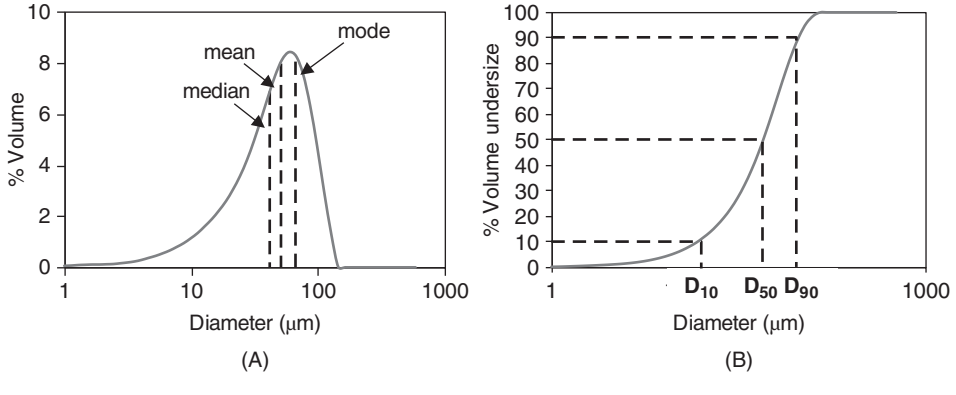


Figure 1.1 *Particle size representations: (A) frequency distribution (non-symmetric); (B) cumulative undersize distribution (with most common percentiles).*

distributions are: number-weighted distributions, resulting from counting techniques such as image analysis, where each particle is given equal weighting irrespective of its size; surface-weighted distribution (normally surface area) where each size is square weighted; volume-weighted distributions, common in static light-scattering techniques, in which the contribution of each particle in the distribution relates to its volume (being equivalent to a mass distribution if the density of the particles is uniform); and intensity-weighted distributions, where the contribution of each particle relates to the intensity of the light scattered by the particle, typical of dynamic light-scattering-based instruments. Number, surface and volume weightings vary as size raised to the zero, second and third powers, respectively. The case of intensity-weighted distributions this is not so simple, and depends on the type of light-scattering device and also on the size range [16] (for example, the intensity of the light scattered by very small particles (<50 nm) is proportional to $[\text{size}]^6$). Figure 1.2 clearly illustrates this point by showing the results of a size distribution of equal numbers of particles with diameters of 5 nm and 50 nm. As expected, the number-weighted distribution gives equal weighting to both types of particles, whereas the intensity-weighted distribution corresponds to a much stronger signal for the coarser 50 nm particles (one million times higher). The volume-weighted distribution is intermediate between the two. This example clearly shows how crucial it is, when reporting particle sizing data, to report not only the size measuring method but also the distribution base. It can then be concluded that different sizing techniques can generate different sizing results for the same sample, not only because different equivalent diameters are being measured, but also because different weighting factors are being used.

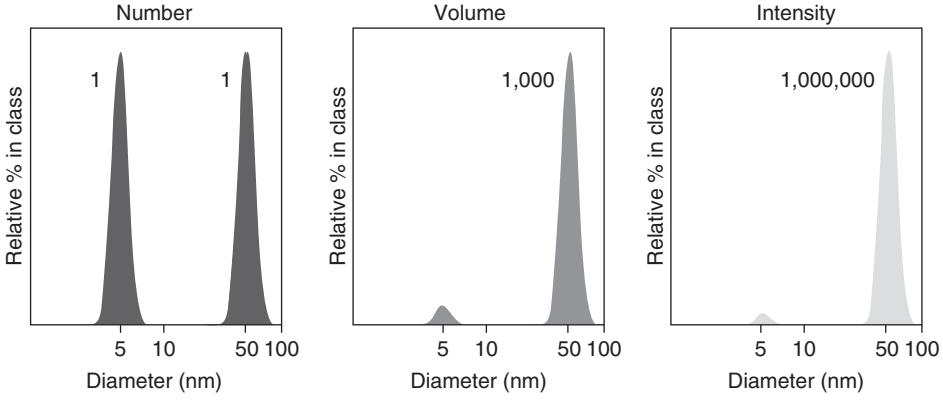


Figure 1.2 *Example of number-, volume- and intensity-weighted particle size distributions for the same sample [reproduced with permission of Malvern Panalytical].*

Volume-weighted (or mass-weighted) particle size distributions are common for most pharmaceutical materials; however, number-weighted representations are useful, for instance, for determining the size of primary particles in agglomerated systems or to detect impurities [3]. Although it is mathematically simple to convert from one type of weighting to another, the converted results are often erroneous [3]. In fact, additional information

about particle characteristics (such as shape factors or optical properties (refractive index)) is normally required for a more reliable conversion, but in general these elements are not available in practice. Thus, whenever possible, a particle sizing technique that gives the desired weighting without transformation should be used.

1.2.3 Distribution Statistics

While a single number cannot describe the size distribution of the sample, sometimes it is tempting to report an "average size" or a central tendency of the distribution along with one or more values to describe the distribution width. A range of statistical parameters can be used for this purpose [16], as for example:

- **mean**: "average" size of a population;
- **median**: size where 50% of the population is below/above – this value is also called D_{50} and is one of the most meaningful parameters for particle size distributions;
- **mode**: size with the highest frequency (highest peak of the distribution), very useful if there is more than one peak in the distribution (multimodal).

For symmetric distributions (also called normal or Gaussian) all these values are numerically equivalent, but for asymmetric distributions with elongated tails, most common in real samples, these parameters correspond to different values, as illustrated in Figure 1.1.

Particular care should be taken with the "mean" values as there are multiple definitions for this parameter related to the basis of the distribution (e.g., number or volume). The various mean calculations are defined in standard documents [23]. Table 1.2 summarizes the most common.

The comparison between two or more particle size data is easier when using the cumulative distribution representations, in the same or separate graphs. Furthermore, in order to

Table 1.2 *Examples of mean diameters*

	Definition	Comment
Number-weighted mean ($D_{1,0}$) (also known as arithmetic mean)	$= \frac{\sum d}{n} = \frac{\sum d^1}{d^0}$	Most common in particle counting applications
Surface-weighted mean ($D_{3,2}$) (also called Sauter mean)	$= \frac{\sum d^3}{\sum d^2}$	Most relevant where specific surface area is important e.g., bioavailability, reactivity, dissolution
Volume-weighted mean ($D_{4,3}$)	$= \frac{\sum d^4}{\sum d^3}$	Most common in instruments where the result is displayed as a volume distribution, most sensitive to the presence of large particles
Intensity-weighted mean (also called Z-average diameter, D_Z, or harmonic mean ($D_{6,5}$))	$= \frac{\sum d_i^6}{\sum d_i^5}$	Most common in DLS for very small particles (Rayleigh scatterers)

quantify the width of the size distributions, it is common to use some parameters of the cumulative curve known as percentiles (D_x where x means the percentage of sample with sizes below this value), typically D_{10}, D_{50} and D_{90}. As mentioned before, D_{50} (the median) is the middle value of the cumulative distribution where the total frequency of values above and below is equal; D_{90} describes the diameter where 90% of the distribution has a smaller particle size than this value (and 10% has a larger particle size); and D_{10} means that 10% of the distribution have diameters lower than this value. These percentiles, easily recognized in a cumulative curve, as previously shown in Figure 1.1, are frequently used to quantify the width (or span) of the size distribution defined as:

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}}$$

Span is normally defined as the distance between two points equally spaced from the median and thus other percentiles can also be used in this definition as, for example, D_{25} and D_{75} (also known as quartiles). Finally, it should be pointed out that cumulative distributions can be represented on linear and logarithmic axes for the particle size (the latter is especially suited for widely distributed data) [16, 18].

1.3 Selecting a Particle Sizer

1.3.1 Classification

As discussed above, the choice of a particle sizer is not an easy task due to several reasons, one of them being the arsenal of particle-sizing technologies and instrumentation currently available on the market, that range from the classical sieves to the more modern and sophisticated light-scattering instruments. As a result of this large variety of methodologies, it is difficult to classify the techniques used for particle size. Nonetheless, some attempts have been made to group them [14, 24, 25]. One criterion is to divide the sizing techniques into *imaging* and *non-imaging*. Imaging techniques obviously allow the direct visualization of the particles and thus can provide, besides size and size range, additional information on particle characteristics like shape, structure, degree of agglomeration and texture, which the non-imaging techniques are unable to give. These methods include optical microscopy/image analysis as well as electron microscopy (SEM and TEM), being mandatory whenever particle shape and structure information is required. They are normally slow and labour-intensive (especially manual microscopy) compared with the non-imaging methods that, on the other hand, are based on the measurement of a particle property related to its size through an equivalent spherical diameter.

Another type of classification is based on the measurement being made one particle at a time, accumulating counts of particles with similar sizes, as opposed to measuring an ensemble of particles at the same time and subsequently extracting the particle size distribution using an appropriate theory (model). The former are called *single particle techniques* (also referred to as *counting techniques*, as particles are individually counted), and typically exhibit high sensitivity and resolution but narrow dynamic size ranges. In contrast, the latter techniques, named *ensemble techniques*, normally have low resolution and low sensitivity but a broader dynamic size range and high statistical accuracy, being better

suited for on-line and in-line applications. A high-resolution instrument can separate two close-together modes, while a low-resolution instrument can only detect one broad peak. Sensitivity in particle sizing can be viewed as a measure of the smallest amount of a given size particle that can be detected by the instrument.

Examples of counting methods are not only the microscopy-based techniques, as image analysis, but the electrozone counters (pioneered by the Coulter company and still known as the Coulter counter technique), the optical counters (optical equivalent to electrozone counters), and the time-of-flight counters targeted at aerosols. In these counting techniques, particles pass individually through the sensing zone (an electrical sensing zone in the case of the Coulter counter, or a photozone in the case of an optical counter) and so very low particle concentrations have to be used in order to avoid coincident effects (i.e., multiple particles being counted together). Another common feature of these methods is that they all need prior calibration, accomplished by using uniform particles of known sizes [16, 17].

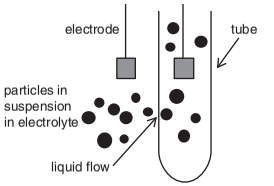
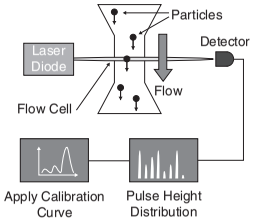
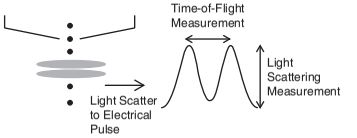
As previously stated, ensemble methods rely on the measurement of a certain property of an ensemble of particles, being the raw detected signal "inverted" mathematically to estimate the particle size distribution of the entire population. For that, the results of these techniques normally depend on the mathematical algorithm used. Examples of ensemble averaging techniques are light-scattering techniques (static and dynamic) and acoustic spectroscopy [16, 17].

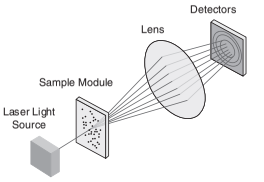
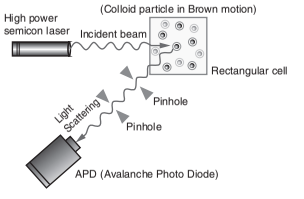
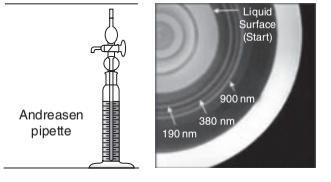
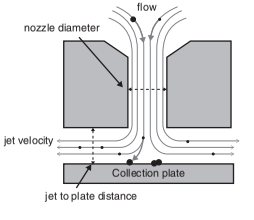
The ensemble methods can also involve fractionation of the samples prior to sizing, in which an outside separation force is applied to the particles, physically separating them according to size (*fractionating methods*). In order to provide a measurement of particle size distribution, the fractionation techniques must be combined with detection techniques, such as optical detection or light attenuation or scattering. Common fractionation techniques are sieves, gravitational sedimentation, differential centrifugal sedimentation, and various forms of particle chromatography [16, 17]. Details of some of these techniques, available on the market and commonly used to characterize pharmaceutical products, can be found in Table 1.3 together with the respective size ranges and the corresponding standards.

1.3.2 Selection Criteria

In making a decision on which technique to use, a number of criteria should be considered [24, 25]. First of all, it is necessary to consider the appropriateness of a particular sizing method to the problem at hand. As explained earlier, different techniques are likely to produce different size results for the same particle, and all of them are likely to be correct. The best instrument for a given application is probably the one that most closely relates particle size to the application of the particles. In other words, the particle property measured by the size analyser should be (whenever possible) related to the end use of the sizing data. Additionally, the measuring principle should be directly related to the weighting mechanism. As also discussed above, the sizing data can be expressed on a different basis (e.g., number, area, volume, light-scattering intensity) depending on the measuring principle. Conversions to another basis, although easily available from the software of most instruments, should be avoided. In the case of pharmaceutical products, a volume-weighted distribution is often the most relevant descriptor of the content of active ingredients as a function of particle size

Table 1.3 *Examples of sizing techniques commonly used in the pharmaceutical industry and respective measuring principles, measured equivalent spherical diameter (ESD) and primary distribution weight, size range and related standards*

Measurement technique	Method/physical principle	Technique layout	ESD/primary distribution weight	Size range (μm)	Standards
Light microscopy/ image analysis	The basic equipment consists of a microscope, a camera and a computer. The image of a dispersed sample is evaluated to assess the shape and size parameters of each particle. This process can be manual or automated.		Projected area diameter/ number weighted	Static: 1–5000 Dynamic: 30–30000	ISO 13322-1:2014 [26] ISO 13322-2:2006 [27]
Electrical zone sensing (Coulter counter)	Particles homogeneously suspended in an electrolyte solution are forced to flow through a small aperture that separates two electrodes of opposite potential. When a particle passes through the aperture, the resistance of the aperture increases, giving a voltage pulse proportional to the particle volume.		Volume diameter/ number and volume weighted	0.5–1500	ISO 13319:2007 [28]
Photo zone sensing (single particle optical sensing: SPOS)	Particles in a liquid suspension are forced between a light source and a detector, producing a shadow or blockage of light on the detector (light obscuration) related to the optical cross-section of the particle.	 [Reproduced with permission of PSS – Particle Sizing Systems]	Optical diameter/ number weighted	0.5–5000	ISO 13099-2:2012 [29]
Time-of-flight	An air stream containing the particles is drawn through a fine nozzle into a partial vacuum producing a supersonic flow of air, causing particles to accelerate according to size.	 Adapted from [50]	Aerodynamic diameter/ number weighted	0.5–20	

Laser diffraction(LD)/ Fraunhofer diffraction (FD)/ low angle laser light scattering (LALLS)	A laser beam is passed through a sample of particles and the scattered light intensity is collected at low angles. The light scattering data is converted into a particle size distribution using Fraunhofer theory.		Fraunhofer diameter/ volume weighted	0.020–2000	ISO 13320:2009 [30] USP 34 NF 29 [31]
Dynamic light scattering (DLS)	The fluctuations of the light scattered by a suspension of submicron particles, due to Brownian motion, are collected over time at a given angle. From the autocorrelation function a diffusion coefficient and an average size is calculated.		Hydrodynamic diameter/light intensity weighted	0.003–3	ISO 22412:2017 [32]
Sedimentation (gravitational and centrifugal)	A sample of particles is uniformly suspended in a fluid and allowed to settle due to gravity according to Stokes' law. Centrifugal sedimentation extends the range of analysis to much smaller particles.		Stokes diameter	Gravitational: 1–250 Centrifugal: 0.01–100	ISO 13317-1:2001 [33] ISO 13318-1:2001 [34]
Cascade impactor	Consists of a number of impactor stages connected in series with smaller and smaller cutoff diameter. Particles having sufficient inertia will impact on a particular stage collection plate, whilst smaller particles with insufficient inertia will remain entrained in the air stream and pass to the next stage where the process is repeated.		Aerodynamic diameter/ mass weighted	0.5–10	USP 26 NF 21 [35]

[1]. However, for studies like, for example, lung toxicity, surface-area-based measurements are indicated [6, 10]. On the other hand, number distributions are, as mentioned before, most suitable for an evaluation of particle contamination or for characterizing fiber-like particles [3].

Another important parameter is the particle size range. The size range of each instrument/technique is normally dictated by the assumptions/equations on which they are based. Many interesting applications involve particles around 1 micron. However this region is apparently a "natural frontier" for many sizing methods [24, 25]. In fact, this size region is considered the dividing line between sedimentation and centrifugation methods (in addition to particle density); Fraunhofer diffraction (for which the scattering pattern is independent of the material refractive index) is also recommended for particles larger than approximately 1 μm while, for submicron particles, dynamic light scattering is more appropriate. Additionally, this size region is regarded as a barrier between light and electronic microscopy. The same applies to electric conductivity and light obscuration based methods for which inconsistent data have been reported for the region 0.5–1 μm [3]. Nonetheless, many manufacturers claim to have increased the operational size range of their instruments below this value by adding some features in the original configuration. This normally results in the production of artefacts in the sizing data, especially at the extremes of the distribution. Thus, the user should be alerted for these details and should look for an instrument whose mid-range covers the size range of interest, that is, avoiding the extremes. Also, for the reasons indicated (namely, differences in the weights of the raw data), the combination of the results of two different sizing datasets in order to cover a larger size range should be avoided.

Other issues to be taken into account when selecting a particle sizer are: the ease of use, the speed, the flexibility, and the cost [24, 25]. Regarding the former, it should be kept in mind that behind the apparent simplicity of the measurement itself, the process of sampling and sample preparation may be complex/laborious. Thus the time of analysis may be just a (small) part of the total time needed. Also the possibility of measuring different types of samples, in a variety of conditions (liquid suspensions or particles in air), that is, the instrument flexibility, is another pertinent parameter, especially if different types of samples have to be measured with the same measuring device. Finally, subjects like accuracy, precision, resolution and sensitivity are also of major relevance in the decision-making process. Accuracy is a measure of how close an experimental result is to the "true" value. This concept is somehow problematic in particle sizing, especially for non-spherical particles for which the "true" values might not be known (but accepted as conventional true values). Precision is the closeness of agreement between a series of measurements from multiple sampling of the same sample. Measurement resolution, mentioned before, is normally defined as the minimum ratio of two monodisperse distributions that can be separated. Resolution and dynamic size range are often inversely related to each other [3]. There are currently many reference materials for checking these parameters.

As a final point, it should be stressed that sampling and dispersion, although of utmost importance in particle sizing, are beyond the scope of this evaluation. In fact, particle size data are only valid when the sample is representative and appropriate dispersion techniques have been used. It is well known that most of the variations in size distribution results are due to incorrect sampling and sample preparation [17, 36, 37].

1.4 Aspects of Some Selected Methods

This section encompasses the discussion of some selected particle sizing methods, probably the most widely applied in the pharmaceutical field. The light microscopy-based methods will be described first as they provide the most direct measurement of particle size and morphology. Next, the incontrovertible light-scattering methods (both static and dynamic), almost universally used, will be discussed. The time-of-flight method and cascade impaction, leading to the measurement of the aerodynamic diameter, of utmost importance to nasal drug formulations, close this selection of methods. It should be pointed out that, rather than describing in detail the operating principles of these methods (easily available in the open literature), this discussion is more focused on their weaknesses and strengths in order to better compare them.

1.4.1 Optical Microscopy-based Methods

Light microscopy-based methods are a powerful tool for particle characterization as they involve the direct observation of the particles and thus can provide information on surface features, besides shape and size, offering a better insight into the nature of the sample. Because of this, they are probably the first choice for investigation of unknown particles, from around 1 micron up to several millimeters. However, manual measurements are lengthy, require skilled operators, are labour-intensive and should be considered qualitative unless a statistically significant number of particles is inspected (statistically representative distributions can be constructed by measuring tens to hundreds of thousands of particles per sample).

Unlike other particle sizing techniques, these methods offer the advantage of providing the particle dimensions of each particle (though two-dimensional) in addition to a series of other shape parameters such as aspect ratio (ratio of the largest to the smallest dimension), circularity (ratio of the actual perimeter of the particle to the perimeter of a circle with the same area), convexity (ratio of the particle area to the total area), along with different geometric diameters, the most common being the projected area equivalent diameter (Table 1.1). Since the particle orientation on a substrate usually gives the maximum area, this leads to an apparently larger size than that measured by other techniques [3]. It is particularly useful for the characterization of particles with extreme shapes (e.g., acicular particles, platy particles, and fibres), as reporting these in terms of a spherical diameter can be, as discussed before, a gross approximation.

Nowadays microscopic methods are normally coupled to image analysis systems that record the data for many particles in very short intervals to be further processed, thus reducing the labour content and minimizing operator bias associated with manual inspection. Image analysis methods can be divided into *static image analysis* and *dynamic image analysis*. The main distinction is whether particles are presented in a static (stationary) orientation or dynamic, that is, flowing past the detector. These systems are currently applied to the characterization of many pharmaceutical products. The basic guidelines for particle size by microscopy image analysis are given in ISO 13322-1:2014, for static image analysis [26], and ISO 13322-2:2006, for dynamic image analysis [27].

Static image analysis uses an optical microscope to characterize particles dispersed on a slide that is moved by an automated stage. Each image is captured by a digital camera and a software routine performs various tasks to distinguish particles from background, separate touching particles, and assign size and shape parameters. The majority of static image analysis measurements are made on powders, typically used for solid oral dosage forms but they may also include suspensions, creams and even aerosols (actuating the device onto a slide) [38, 39]. The size range is typically between 1 μm and 1 mm.

Dynamic image analysis measures a stream of flowing particles using a rapid strobe light on one side of the stream and a digital camera on the other capturing the particle images used in the analysis. In this case, the sample is transported either by gravity, air pressure, or in a liquid which passes the camera, and pictures are taken in quick succession, giving tens of thousands of images per minute. Because of the size range measured by dynamic image analysis (30–30 000 μm) they have been related to sieve results with the bonus of shape information [38]. Modern systems use a hydrodynamic sheath flow mechanism to efficiently focus the particles [21], and others use two cameras with different magnifications to cover a wide measuring range, as illustrated in Figure 1.3.

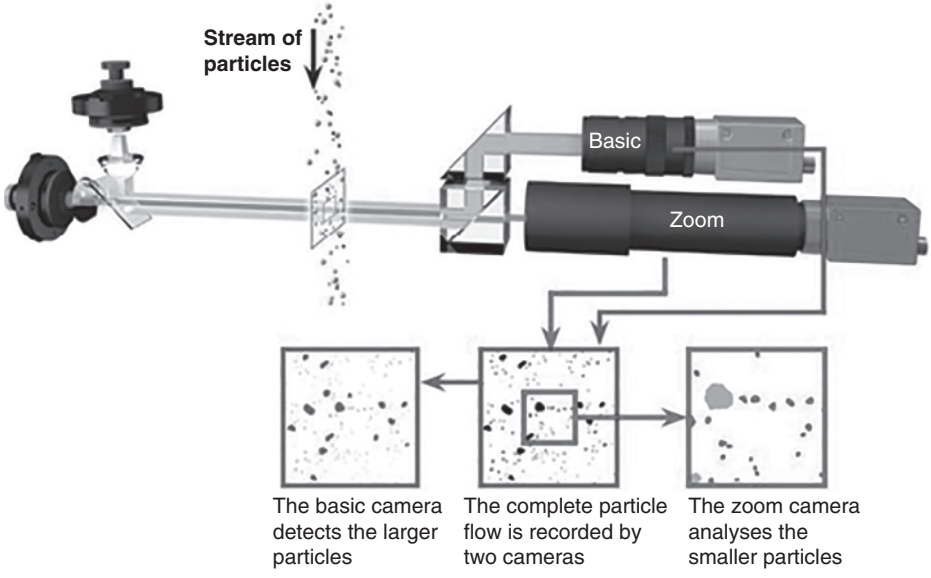


Figure 1.3 *Measuring principle of dynamic image analysis [reproduced with permission of Retsch Technology].*

Since image analysis is a high-resolution counting technique, it can effectively detect outlier populations (both larger and smaller than the main population). For active pharmaceutical ingredients this could be a great advantage for finding small amounts of particles that could negatively impact dose uniformity [38, 39].

It should be remembered that image analysis, as a counting method, generates number-based distributions. Volume-weighted distributions can be calculated assuming that the particles are spherical and of uniform density which, for particles deviating from

sphericity, can lead to false results if adequate shape factors are not applied. Finally, it should be pointed out that this technique is often used in conjunction with ensemble-based particle sizing methods, such as laser diffraction, to gain a deeper understanding of the sample or to validate the ensemble-based measurements [7, 39, 40].

1.4.2 Laser Light-scattering Techniques

Laser scattering-based particle characterization techniques are probably the most popular for sizing particles as they are fast, easy to use, flexible (enabling the measurement of particles in liquids as well as in sprays, aerosols, emulsions and dry powders), highly repeatable, and have a wide dynamic size range (from a few tens of nanometers up to several millimeters). Additionally, these are well established and standardized techniques (ISO 13320:2009 [30] and USP 34 NF 29 [31] for laser diffraction, and ISO 22412:2017 [32] for dynamic light scattering).

In the pharmaceutical industry they are becoming a preferred standard method [41, 42]. However, they have been the subject of considerable criticism, mainly because some working parameters that are decisive for a correct size analysis are not always observed [3, 43]. In fact, although it is a very user-friendly technique, laser-scattering techniques can lead to erroneous data if not used properly. To avoid that, the users must have a sound knowledge of its basic principles and limitations. Additionally, the lack of definition (and misuse) of some terms and acronyms, indistinctly used in many situations, also entails some confusion. The next paragraphs will briefly mention the fundamentals of this technique, not only to clarify the meaning of those acronyms, but also to explain the reasons why some precautions should be taken to ensure a proper analysis and the consequences when they are not taken. Both methodologies of light scattering (static and dynamic) are included in this section.

Light scattering is the alteration of the direction and intensity of the light beam that strikes an object, due to the combined effects of reflection, refraction and diffraction (diffraction is the bending of light at the particle's boundary), as illustrated in Figure 1.4, creating a complex pattern known as "scattering pattern". More specifically, a scattering pattern is

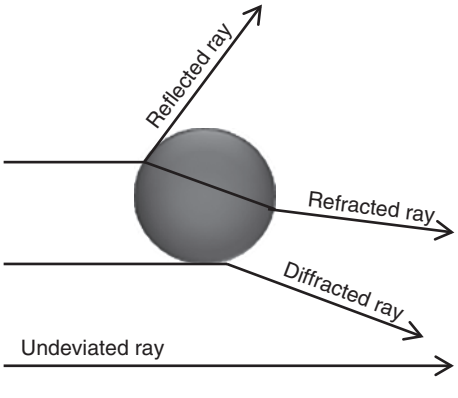


Figure 1.4 Interaction of light rays with a particle.

formed by light intensity as a function of the scattering angle (measured relative to the direction of the incident light) [16, 17, 44, 45].

The intensity of the scattered light is basically a function of the incident wavelength, the scattering angle, the particle size and shape, and the ratio of refractive indices of the particle to that of the medium. The shape of the scattering pattern is highly dependent on the ratio of the particle size to the incident wavelength, as illustrated in Figure 1.5. From this figure it is clear that particles larger than the wavelength of the incident light will scatter light essentially in a forwards direction, and as the particle size decreases the relative intensity of the backward scattered light increases compared to the forward light lobe, being approximately symmetric for particles much smaller than the wavelength (Rayleigh scatterers). This fact enables the measurement of particles of different sizes using different methodologies.

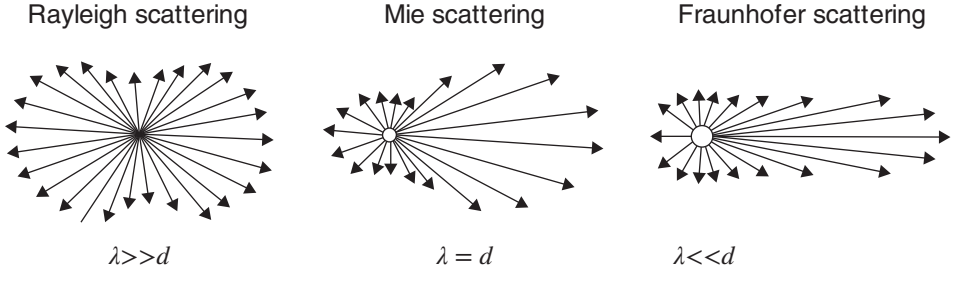


Figure 1.5 *Effect of particle size (d) on the scattering pattern for a given light wavelength (λ).*

Although generally called "scattering techniques", these techniques can be divided into two groups whose working principles are entirely different: static light scattering (SLS) and dynamic light scattering (DLS). In SLS, particle size information is extracted from the scattering pattern obtained when the sample material is illuminated with a light beam; the scattered light intensity averaged over a given time interval at various scattering angles (scattering pattern) is then used to derive particle size information, based on a suitable scattering theory (model). On the other hand, DLS records the fluctuations in the intensity of the light scattered by the particles as a function of time, measured at a fixed angle in order to calculate the particles' diffusion coefficient, which in turn can be used to determine their hydrodynamic radius. This methodology is recommended for submicron particles, including nanoparticles. Both of these techniques will be discussed in more detail below.

1.4.2.1 Laser Diffraction and Static Light Scattering

Included in the first group (SLS) is the popular laser diffraction (LD) technique. Although "laser diffraction" and "static light scattering" are often used interchangeably to refer to the same particle size determination, this is not correct as discussed next. As illustrated in Figure 1.5, large particles (i.e., with diameters considerably larger than the wavelength of the incident light) scatter light mainly in the forwards direction, being the scattering pattern dominated by diffraction phenomena. In a typical experiment of laser diffraction, a representative sample is passed through a collimated beam of monochromatic light (usually a laser) and the light scattered by the particles at various angles (scattering pattern) is

measured in a multi-element detector (see technique layout in Table 1.3) and subsequently transformed into a particle size distribution. The method relies on the fact that diffraction angle is inversely proportional to particle size. However, it should be pointed out that the scattering pattern of an ensemble of particles is a composite pattern resulting from the contributions of all the individual particles (i.e., the resulting light at each point is the sum of the contribution of each individual pattern). These patterns are said to be *convoluted*. In the case of laser diffraction, the deconvolution of the scattering data into a volumetric size distribution is based on the Fraunhofer theory [16, 17, 44, 45]. This is accomplished by an iterative process of fitting the theoretical model to the data until the two converge within an acceptable amount defined by the manufacturer. In Fraunhofer theory, the refractive index of the particles is irrelevant, which is very convenient (namely when characterizing mixed or unknown powders) since normally this parameter is not easily obtained. Because of this, LD is also called Fraunhofer diffraction (FD). Furthermore, because information about larger particles is contained in low scattering angles (<35°), this technique is also named low-angle laser light scattering (LALLS). The variety of acronyms may be confusing to the less familiar user.

It should be kept in mind that Fraunhofer scattering is only valid for large particles, that is, particles that are at least 5–10 times larger than the incident wavelength, which limits the application of LD to particles larger than a few tens of micrometers. For smaller particle sizes (similar or smaller than the incident wavelength), the light is increasingly scattered with large angles (Figure 1.5), and the scattering pattern is not the result of diffraction phenomena exclusively. Thus Fraunhofer theory is no longer valid and a more complex theory, Mie theory, has to be used to account for all possible interactions between particles and light, yet it is only applicable to spheres [16, 17, 44, 45]. Figure 1.6 illustrates the discrepancy in the size distribution curves resulting from applying Fraunhofer and Mie theories to invert the scattering data obtained for a 6 μm latex standard (larger and/or more opaque particles lead to more convergent results).

Besides being much more complex than Fraunhofer approximation, from the mathematical point of view, the Mie formula requires information about the particle optical properties,

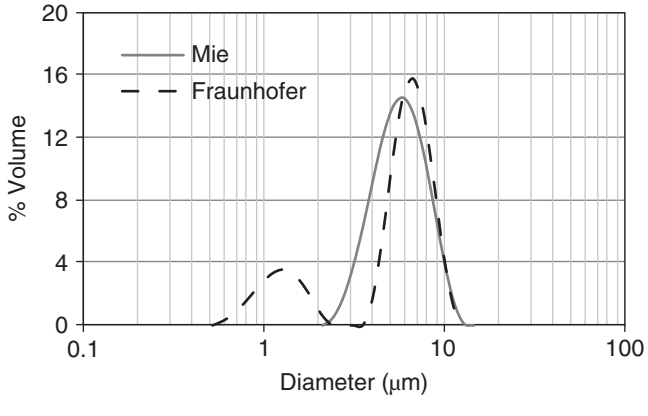


Figure 1.6 *Influence of the scattering model used in the deconvolution of the scattering data: Fraunhofer vs. Mie.*

such as refractive index (real refractive index and imaginary refractive index). This is a major drawback as these parameters are difficult to obtain in practice (especially the imaginary part), and errors in the estimation of the refractive index can give radically different particle size distributions mainly for particles smaller than approximately 10 μm [3, 40, 41, 46]. Most of the currently commercialized analyzers possess a databank with information on refractive indices of various materials and also provide ways to guess them based upon an iterative procedure to fit the modeled data to the actual data [21, 38].

Additionally, the intensity of the scattered light also decreases with particle size and thus the intensity of the signal collected by the detector also decreases (as mentioned earlier, scattered light intensity of very small particles (<50 nm) is proportional to the sixth power of the diameter). Depending on the incident wavelength, the minimum particle size theoretically possible would be around 500 nm. Nonetheless, the instruments in use nowadays claim to measure particles down to 20 nm. To extend the measuring range down to this level, additional techniques have to be combined with laser diffraction. These include the use of more than one laser (shorter wavelengths), supplementary detector arrays (located sideways and even backwards), different light polarizations (PIDS), additional lenses, and application of different inversion algorithms, among others [3, 43]. Figure 1.7 shows a configuration where, besides the ring-shaped detector located at small scattering angles (for larger particle measurement), additional large angle and backscatter detectors are used to allow the measurement of smaller particles.

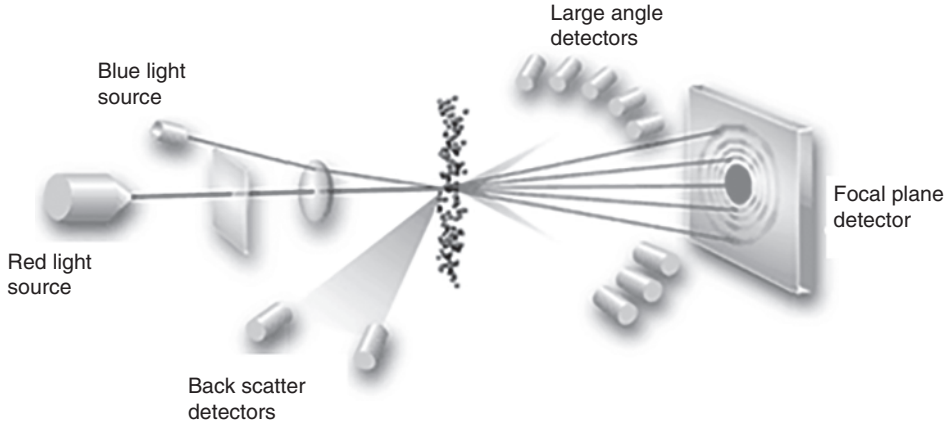


Figure 1.7 *Schematic representation of the static light scattering technique using different lights and rear detectors [reproduced with permission of Malvern Panalytical].*

These modifications, carried out in a diverse way by different equipment manufacturers, are responsible for many discrepancies in the sizing results that would not be expected when using "in principle" the same technique, and may justify some of the criticisms that have been directed to these techniques. In conclusion, modern laser "diffractometers" do not rely simply on laser diffractometry but include additional technologies to extend the measuring range to submicron particles. Therefore this technique, erroneously called "laser diffractometry", is indeed static light scattering [3, 43].

Major drawbacks of this technique are: the low resolving power (typical of ensemble methods), the fact that the result accuracy is also dependent on the accuracy of the optical parameters used, as explained, and not being recommended for particles with extreme aspect ratios [47]. Caution should also be taken when comparing laser diffraction data with other methods, as the Fraunhofer diameter is not a volume-equivalent diameter (it is associated with an average projected area diameter) [3, 20, 40]. Additionally, these techniques are normally applied to relatively low concentrations in order to avoid multiple scattering (where light scattered from one particle is scattered by a second particle before reaching the detector). Nonetheless, it continues to be very popular and one of the first options for general purposes.

1.4.2.2 Dynamic Light Scattering

Dynamic light scattering (DLS) is also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS) (because when photons are scattered by mobile particles, the process is quasi-elastic). It is a non-invasive, user-friendly method used since 1960 to routinely size submicron particles (from a few nanometers to a few micrometers, these limits being sample-dependent), yielding very accurate results especially for monomodal samples, in very short times, with low sample preparation requirements. It is an absolute technique as there are no adjustable parameters for a "calibration", in contrast to microscopes or particle counters. It has been widely used in the characterization of proteins, biomaterials, liposomes, micelles, colloidal dispersions and emulsions [32, 48].

This method is based on the determination of the hydrodynamic properties of submicron particles suspended in a liquid medium. If the particles are very small, their collisions with the solvent molecules result in an erratic motion called Brownian motion. When these particles are illuminated by a laser, the intensity of the scattered light measured by a single detector fluctuates as a result of the Brownian movements, which are indirectly proportional to size. The time dependence of the intensity fluctuations is analyzed with a very sensitive detector (as the intensity of the light scattered by very small particles is very low), normally a photomultiplier positioned at a suitable angle (either 90° or 175°) (see technique layout in Table 1.3). The signal of the photomultiplier is fed to a correlator to build the so-called "autocorrelation function" (which led to the acronym PCS), which is basically an exponential decay curve whose decay rate is related to the translational diffusion coefficient of the particles. This coefficient can be transformed into an average diameter (hydrodynamic diameter, Table 1.1), using the Stokes–Einstein equation. The hydrodynamic diameter is very similar to the geometric diameter, with the exception of very small (<300 nm) highly charged particles where the electric double layer gives a larger diameter than that measured by microscopy [40].

The autocorrelation function of polydisperse samples is a summation of single exponential functions (each corresponding to a different size) that, similarly to laser diffraction, needs to be deconvoluted to derive the corresponding particle size distribution. Different deconvolution algorithms have been applied for this purpose, depending on the type of information required. Normally a mean particle size (Z average diameter, Table 1.2) and the width of the distribution are sufficient for monomodal samples. The interpretation of data from polydisperse samples is considerably more difficult and requires the use of more complex algorithms that do not always have simple or unique solutions [48].

It should be pointed out that the size distribution generated by DLS is an intensity-based distribution. However, it can be converted to a volume-based distribution using Mie theory, which, as mentioned above, requires *a priori* knowledge of the particles' optical properties. One of the major drawbacks of this technique is the sensitivity to the presence of larger particles in the sample. As explained earlier, for this size range, light intensity is proportional to the sixth power of particle diameter, and thus the existence of a few larger particles can dominate the particle size result [40, 49]. In this case, complementary measures using laser diffraction are recommended.

The limitation of this technique to dilute suspensions has been partially overcome by new instrumentation configurations that have been developed (namely using backscattered detection). Finally, DLS is (as laser diffraction) a low-resolution method and, generally, can only resolve particle populations that differ by a factor larger than 2:1 [48]. Nonetheless, DLS is very good, quick and accurate for sizing narrow distributions of colloidal particles.

1.4.3 The Time-of-Flight Counter

This technique was developed in the late 1980s and is currently commercialized by TSI as the Aerodynamic Particle Sizer (APS), a third-generation instrument [50]. It is a fast stream scanning technique with high resolution, able to count and measure the size of aerosols between 0.5 and 20 μm in real time. Although the particle size distributions of pharmaceutical sprays and aerosols have traditionally been undertaken by methods involving multi-stage cascade impactors, these are slow and labour-intensive and other options have been adopted, namely the time-of-flight (TOF) method because of its ability to measure very small sample quantities and to count hundreds of thousands of particles in a very short time, and also because of its size-resolving capability [39]. The ability to determine the aerodynamic particle size (Table 1.1) is of particular importance, for instance to characterize orally inhaled dosage forms, as the knowledge of this parameter is quite useful for predicting, for example, the regional deposition of the particles in the human respiratory tract.

Size is measured as follows: after passing a dilution stage, incoming particles are accelerated singly through a well-defined flow field, in which particles experience ultra-Stokesian acceleration. Particles accelerate in the airflow according to size (smaller particles accelerating more rapidly than larger particles due to differences in mass). After the acceleration nozzle, particles pass into the sensing zone, where they are sized according to their transit time through two partially overlapping laser beams (see technique layout in Table 1.3). The first laser beam detects each particle and starts a TOF clock, while arrival at the second laser stops the clock. The transit time of the particles between two laser beams is a monotonic function of size. The aerodynamic diameter is calculated from calibration curves using spherical particles of known density. The equipment is easy to operate and maintain, but some problems related to the optimization of the analysis conditions remain to be solved [39, 51, 52].

The count-weighted size distribution can be transformed to a mass-weighted basis, assuming the particles are spherical and of uniform density. The size distribution can be biased when the drug-free droplets are mixed with the drug particles [53]. Recent versions of this equipment have minimized coincidence effects and extended the dynamic size

range of the older models [3]. It has been considered adequate for aqueous droplet-based formulations, but for droplets larger than 5 µm, some distortion can occur. Also for non-spherical particles the results can be underestimated [3, 39]. The main limitation, compared with cascade impaction, is the lack of chemical assay of the active pharmaceutical ingredients, and for this reason it is not suitable for mixtures of active components and excipients.

1.4.4 Cascade Impactor

Inhalation products, namely nebulizers and dry and metered dose inhalers, represent a significant proportion of pharmaceutical products. The optimal design of dry powder inhalers is, in particular, challenging [14]. As mentioned above, aerodynamic diameter is a key parameter for these products, and thus its measurement is critical either during the product development cycle or for quality control.

From the various techniques available, multiple stage cascade impaction is the most widely used method, being the standard technique recommended by regulatory bodies [35] as it allows the analysis of mass-weighted aerodynamic particle size directly together with the capability for recovery and assay for active pharmaceutical ingredients in a traceable manner [54]. Alternative methods, such as TOF, that also measure aerodynamic particle diameters, do not provide (at least in their basic configuration) differentiation between active pharmaceutical ingredients and any other components in the formulation, and only deliver a number-based particle size distribution.

Cascade impactors are based on inertial separation (function of particle size and velocity) and allow direct measurement of mass-weighted aerodynamic particle size of drug substance in aerosols between 0.5 and 10 µm. These devices consist of a series of stages, each comprising a plate with a specific nozzle and a collection surface (see technique layout in Table 1.3). Sample-laden air is drawn into the impactor, flowing sequentially through the stages. As particles pass through the nozzle, they either remain in the air stream or impact on the collection surface. Particles with sufficient inertia are collected, while the rest pass on to the next stage. Each deposition stage provides a defined aerodynamic cutoff diameter (particles collected at 50% efficiency). As nozzle size decreases with increasing stage number, particle velocity increases as they proceed through the instrument, allowing the collection of increasingly small particles. The sample is thus separated into a series of size fractions, individually collected for subsequent analysis by HPLC (high pressure liquid chromatography) to determine the amount of active compound collected at each stage. The most widely used cascade impactors, the Andersen Cascade Impactor and the Next Generation Impactors, separate a sample into 7–8 discrete size fractions.

Nonetheless, cascade impactor determinations are rather time-consuming and complex to undertake, also being unsuitable for making size-resolved measurements of large liquid droplets as found in nasal sprays (20–200 µm). Thus real-time techniques, not only TOF instruments but also static light scattering and microscopy-automated image analysis (especially when combined with Raman chemical imaging), have been considered potential options for the rapid assessment of particle size in aerosols, especially in early-phase product development [39]. Additionally, these techniques are simple to perform and enable auto-

matic data recording and processing. With regard to static light scattering, this technique is, as mentioned earlier, very versatile and enables the analysis of the aerosol by directly spraying it into the laser beam [41]. Equipment producers nowadays offer the possibility of *in situ* analysis of high concentration aerosols and sprays, using a so-called inhalation cell [55]. Several studies have been published comparing the multi-stage cascade impactor and laser diffraction systems [41, 56]. Despite the results not always being consistent with the cascade impactor, namely due to propellant evaporation and droplet break-up problems, and also because light scattering techniques deliver volume-equivalent diameters, the rapidity of measurement, the fact of being a non-invasive technique, the size-resolving capability and the much wider dynamic size range (up to 2 mm) are important points that make SLS a valuable tool for rapid screening [53]. However, in many instances, particularly with aerosols from aqueous solutions, aerodynamic and other physical diameters determined by microscopy and light scattering converge because the droplets are spherical and have unit density [39].

1.5 Conclusions

In the pharmaceutical field, particle size distribution should not only be known but should be controlled. However, the choice of a particle sizer is a more complex task than it seems. Indeed, from this chapter it is clear that there are no universal sizing techniques suitable for all samples, and that no technique can perform measurements from zero to infinity. Moreover, the wide diversity of instruments, based on different measuring principles, will generate different sizing data. Explanations for all these aspects were addressed above, including several points that need to be taken into account when choosing particle sizing equipment for a given application. Making the correct choice requires not only knowledge of the instrument working principle, but also of the type of equivalent diameter measured and the size distribution weight. This information has to be compatible with the analysis end-use and the particle properties.

A detailed discussion of some methods currently used for routine analysis of pharmaceutical products clearly indicated that they all exhibit advantages and limitations. Imaging techniques are most useful to visualize the particles, as well as to assess information about shape and structural parameters. Nonetheless, they suffer in general from statistical problems. On the other hand, ensemble techniques like the light-scattering techniques are more rapid and versatile, but involve deconvolution processes that limit resolution, requiring in most cases information about the particles' optical properties (not easily available). As for the measurement of the aerodynamic diameter, alternatives to cascade impaction have recently been considered.

The overall conclusion is, however, that a comparison of methods, yielding complementary information, is highly recommended.

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