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Determination of particle size distribution — Electrical sensing zone method —

Part 1: Aperture/orifice tube method

Détermination de la distribution granulométrique — Méthode de détection de zones électrosensibles —

Partie 1: Méthode d'ouverture/d'orifice du tube

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 24, *Particle characterization including sieving*, Subcommittee SC 4, *Particle characterization.*

This first edition of ISO 13319-1 cancels and replaces ISO 13319:2007, which has been technically revised. The main changes compared to the previous edition are as follows:

— a general update to reflect the needs of modern quality assurance;

— the section on repeatability and inter system variation has been expanded;

— many instruments of this type are under strict controls within the pharmaceutical and related industries, therefore a new annex has been prepared with details of the factors which should be considered when developing a validated method in this arena;

— Clause 10 now gives details of the exact parameters which should be reported, in order to present the method and the key parameters of the result.

A list of all parts in the ISO 13319 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Determination of particle size distribution — Electrical sensing zone method —

Part 1: Aperture/orifice tube method

1 Scope

This document specifies the measurement of the size distribution of particles dispersed in an electrolyte solution using the electrical sensing zone method. This can include biologics such as cells, but also industrial particles such as carbon, cement, ceramic powders, metal powders, pigments and polymer powders. The method measures pulse heights and their relationship to particle volumes or diameters, and is applicable over the range (implementation dependant) from approximately 0,5 µm to above 1 mm. This document does not address the specific requirements of the measurement of specific materials.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
dead time
time during which the electronics are not able to detect particles due to the signal processing of a previous pulse

3.2
aperture
small diameter hole through which suspension is drawn

3.3
sensing zone
volume of electrolyte solution within, and around, the aperture in which a particle is detected

3.4
analysis volume
volume of suspension that is analysed

3.5
size bin
size interval to distinguish particle size for size distribution measurement

3.6
envelope size
external size of a particle as seen in a microscope

3.7
envelope volume
volume of the envelope given by the three-dimensional boundary of the particle to the surrounding medium

3.8
effective density
density of a porous particle where open pores are filled with liquid and closed pores are not (so included in the density)

4 Symbols

For the purposes of this document, the following symbols apply.

A_p	amplitude of the most frequent pulse
A_x	amplitude of the electrical pulse generated by an arbitrary particle
D	aperture diameter
d_m	certified mean diameter of the microspheres used for primary calibration
d_{micr}	mean diameter of the sieved fraction as determined using microscopy
d_{ESZ}	mean diameter of the sieved fraction as determined using the ESZ instrument
df	degrees of freedom
d_L	particle diameter at the lower boundary of a size interval or channel
d_p	modal diameter of a certified particle size reference material
d_{ref}	reference diameter of the microspheres
d_U	particle diameter at the upper boundary of a size interval or channel
f_{resp}	response factor
K_d	calibration constant of diameter
K_{da}	arbitrary calibration constant of diameter of any value to start the mass calibration procedure
m	mass of sample
M_b	mass balance, percentage of particles accounted for in a measurement in comparison to input particle mass
M_m	mass of particles measured by the instrument
n	number used to signify the maximum of an integral be it channel number [in Formula (D.1)] or number of repeat measurements [in Formulae (G.1) and (G.2)]
n_c	counted particle number
N_c	mean of a Poisson distribution, used to describe the temporal spread of counts within a size bin

N_{c5} count for 5 % coincidence

N_i total number of counts across all size intervals

ΔN_i number of counts in a size interval i

$\overline{N}$ mean of particles in counts N_i (i = 1, 2, 3…n)

p significance level of statistical test

V_m analysis volume

V_T volume of electrolyte solution in which a mass, m is dispersed

$\overline{V}_i$ arithmetic mean volume for a particular size interval i

x diameter of a sphere with volume equivalent to that of the particle

x_{max} maximum particle size that can be obtained on a specific aperture

x_{min} minimum particle size that can be obtained on a specific aperture

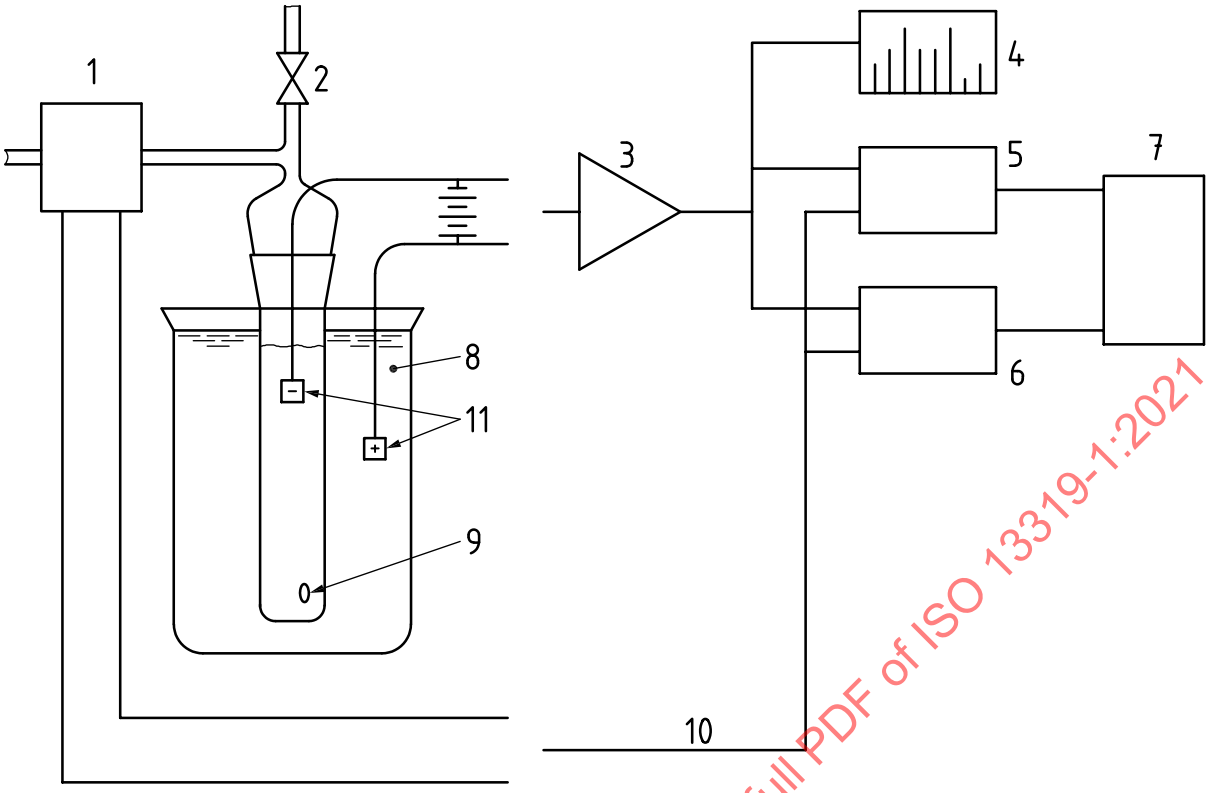
ρ immersed density/effective density (solid density including eventual closed pores, but excluding open pores within the particles)

χ^2 chi-squared statistical distribution

5 Principle

A dilute suspension of particles dispersed in an electrolyte solution is stirred to provide a homogeneous mixture and is drawn through an aperture in an insulating wall. An electric current applied across two electrodes, placed on each side of the aperture, enables the particles to be sensed by the electrical impedance changes as they pass through the aperture. The impedance pulses generated by particle passage are amplified and digitally captured, and the pulse height and shape are analysed, yielding particle count data. The pulse height is regarded directly proportional to particle volume. After employing a calibration factor, a distribution of the number of particles against the volume-equivalent diameter is obtained. The size range of particles to be measured depends upon the size of the aperture.

Conventionally, particles having a size greater than around 0,5 µm are measured by the technique. A schematic of the instrumentation is given in Figure 1.



Key

1	volumetric metering device	7	output
2	valve	8	stirred suspension of particles in electrolyte solution
3	pulse amplifier	9	aperture
4	oscilloscope pulse display	10	counter start/stop triggered by the volumetric device
5	counting circuit	11	electrodes
6	pulse-height analyser		

Figure 1 — Diagram illustrating the principle of the electrical sensing zone orifice/tube method

6 General operation

6.1 Response

The response (i.e. the electrical pulse height generated when a particle passes through the aperture) has been found both experimentally and theoretically to be proportional to the particle volume if the particles are spherical [1]-[3]. This has also been shown to be true for particles of other shapes; however, the constant of proportionality (i.e. the instrument's calibration constant) may be different [4]. In general, particles should have a low conductivity with respect to the electrolyte solution, but particles with high conductivity can be measured e.g. metals [5], carbon [6], silicon and many types of cells and organisms, such as blood cells [7],[8]. For porous particles, the response may vary with the porosity [9],[10]. Recommendations for the measurement of conducting particles and porous particles are given in Annex E.

As the response is proportional to the volume of particles, the pulse amplitude provides a relative scale of particle volumes. By calibration, this scale may be converted to spherical diameter. The calibration constant based on diameter may be calculated by Formula (1):

$$K_{\mathrm{d}} = \frac{d_{\mathrm{p}}}{\sqrt[3]{A_{\mathrm{p}}}} \tag{1}$$

The size, x, of any particle can be calculated by Formula (2):

$$x = K_{\mathrm{d}} \cdot \sqrt[3]{A_x} \tag{2}$$

Typical apertures have a length to diameter ratio of 0,75. This causes some variation in the electrical field within the aperture, which leads in turn to some deviations in the particle sizes measured. This can be countered by increasing the aperture length.

6.2 Size limits

The lower size limit of the electrical sensing zone method is generally considered to be restricted only by thermal and electronic noise. It is normally stated to be about 0,6 µm but, under favourable conditions, 0,4 µm is possible. There is no theoretical upper size limit, and for particles having a density similar to that of the electrolyte solution, the largest aperture available (normally 2 000 µm) may be used. The practical upper size limit is about 1 200 µm, limited by particle density.

The size range for a single aperture is related to the aperture diameter, *D*. The response has been found to depend linearly in volume on *D*, within about 3 % under optimum conditions, over a range from 0,015 *D* to 0,8 *D* (i.e. 1,5 µm to 80 µm for a 100 µm aperture) although the aperture may become prone to blockage at particle sizes below the maximum size where the particles are non-spherical. In practice, the lower limitation is due to thermal and electronic noise and the upper limitation is due to non-spherical particles passing through the aperture. This restricts the operating range to be within 2 % to 60 % of the aperture size. This size range can be extended by using two or more apertures (see Annex F). In practice, this procedure can be avoided by the careful selection of the diameter of one aperture, to achieve an acceptable range.

Sedimentation of particles becomes important when the particles are large and have a high density (for example, 100 µm quartz particles have a sedimentation rate in water of about 1 cm/s). Large apertures are available, up to 2 000 µm. In such applications, the viscosity and the density of the electrolyte solution should be increased, for example, by addition of glycerol or sucrose, in order to prevent particle sedimentation and to increase the possibility of keeping the particles in homogeneous suspension. The homogeneity may be checked by repeated analyses at a range of stirrer speeds. The results of this should be compared to establish the lowest stirrer speed at which recovery of the largest particles is maintained.

6.3 Effect of coincident particle passage

Ideal data would result if all particles traversed the aperture singly and, thus, would produce single pulses. However, the opportunity exists, especially at increased concentrations, that two or more particles arrive in the sensing zone more or less together, which would result in a complex pulse.

Several possibilities exist, i.e (a) two particles pass the sensing zone at the same time, leading to a pulse height equal to the sum of both pulse heights, and to a loss of counts; (b) two particles pass the sensing zone at slightly different times but within the same measurement period of the larger particle, leading to the same pulse height for the larger particle but a distorted pulse shape, and to a loss of counts; (c) two particles, which are individually too small for measurement but have together sufficient volume, pass the sensing zone at the same time, leading to an extra pulse of measurable height, and to an increase of counts. This occurrence is named coincidence. Its effects will distort the size distribution obtained but can be minimized by using low particle concentrations. The probability of coincidence may be described by a Poisson distribution (see Annex A). Table 1 shows counts per millilitre for the

coincidence probability to be 5 % as well as the corresponding analysis volumes to count 100 000 particles.

Table 1 — Counts for 5 % coincidence probability and analysis volumes for 100 000 counts

Aperture diameter D µm	Maximum counts for 5 % coincidence[a] $N_{5\,\%}$ #/ml	Analysis volume for 10^5 counts[b] V_a ml
1 000	5,0E + 01	2 000
560	2,8E + 02	351
400	7,8E + 02	128
280	2,3E + 03	44
200	6,3E + 03	16
140	1,8E + 04	5,5
100	5,0E + 04	2
70	1,5E + 05	0,69
50	4,0E + 05	0,25
30	1,0E + 06	5,4E - 02
20	6,3E + 06	1,6E - 02
10	5,0E + 07	2,0E - 03

[a] Calculated using formula $N_{5\,\%} = 5{\cdot}10^{10}/D^3$ particles per ml.

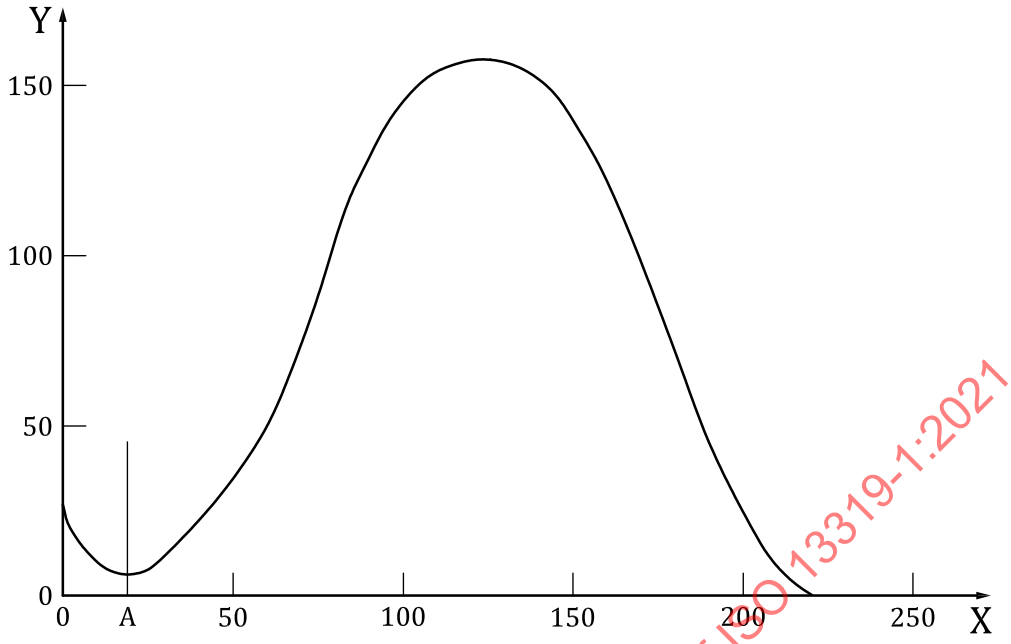
[b] Use pro rata values for other analysis volumes and count numbers.

Counts per millilitre should always be less than these quoted values. Since particle size distributions should not be a function of concentration, the effect of coincidence can be tested by obtaining a distribution at one concentration and comparing it with that obtained when the concentration is halved. In such a test, repeat such dilutions until the reduction in count in a channel with the largest number decreases in proportion to the dilution. This should always be done when analysing very narrow size distributions, as this is where the effect of coincidence is most noticeable.

6.4 Dead time

In instruments using digital pulse processing routines, the signal is scanned at high frequency. Information on pulse parameters, such as maximum pulse height, maximum pulse width, mid-pulse height, mid-pulse width and pulse area is stored for subsequent analysis. In this case, analogue-to-digital conversion of the pulse with storage of the size value for the pulse is not performed in real time and dead time losses are avoided.

To minimize the effect of dead time, the analyser should be used with the lower threshold set to exclude thermal and electronic noise, as indicated at A in Figure 2. Additionally, the concentration of particles should be maintained below 5 % coincidence levels.



Key

X channels

Y counts

NOTE Counts at channels below A are noise counts. True particle counts are at the higher channels.

Figure 2 — Typical results

6.5 Analysis volume

The analysis volume should be chosen based on the following requirements:

a) allow a representative sample of the suspension;

b) allow a sufficient number of particles to be counted and measured in relation to the required quality of the size distribution; and

c) have sufficient precision for the number of particles to be counted if particle concentration is of interest.

Typical values of the analysis volume are given in Table 1.

Table 1 shows that the analysis volumes become excessive for counting this particle number when the aperture diameter becomes greater than 140 µm. Then, counting less particles means that less information on the size distribution will become available, so consideration should be taken into taking a representative sample.

7 Repeatability and reproducibility of counts

7.1 Instrument repeatability

In a correctly performed analysis, the number of counts in a size interval is a random variable which follows a Poisson distribution. In this, the variance is equal to the expected (mean) value. This indicates that the standard deviation of a number of counts, n_c, with mean, N_c, approximates to $\sqrt{N_c}$. Both the variance and the standard deviation can be used in statistical tests on the correctness of instrument operation or sample preparation. The statistical chi-squared test can be used to test whether obtained

data follows a Poisson distribution or not. In this, the apparent and the theoretical variance for a given number of measurements and a given probability are related. An example is given in Annex G. This statistical test can be performed on single size intervals, groups of size intervals, or on the total particle count.

7.2 Method reproducibility/intermediate precision

The reproducibility and intermediate precision will be influenced by several factors (in addition to those dealt with in 7.1). They are covered in detail in Clause 8, specifically in 8.3, 8.4 and 8.11.

8 Operational procedures

8.1 General

A summary of all the key factors that can influence the quality of the final result is given in Annex B. This could be used as the basis for setting a method in accordance with the theory of "quality by design", where the variance (or lack of variance) of these factors on the final result is considered as part of method development and validation and a control structure is put in place for the critical parameters.

8.2 Instrument location

The instrument should be sited in a clean environment that is free from electrical interference and vibration. If organic solvents are to be used, the area should be well ventilated.

8.3 Linearity of the aperture/amplifier system

The linearity of the aperture/amplifier system can be checked using three materials consisting of near mono-sized particles with a certified modal diameter. In a suitable electrolyte solution, the instrument is calibrated with particles at about 0,3 D (see 8.11.2). Two further sizes of particles are then added to the suspension, one size in the range 0.05 – 0,1 D and one size about 0,5 D. The suspension is re-analysed and the size corresponding to these extra peaks should correspond to the quoted size of the particles to within 5 %.

8.4 Linearity of the counting system

The linearity of the counting system can be tested by obtaining three repeat measurements of the total counts (across all channels) at an arbitrary concentration. The concentration is then reduced and three further repeat total counts obtained. Coincidence-corrected counts shall be used. The ratio of the mean of the total counts should be the same as the dilution. If the agreement is not within 5 %, the test should be repeated comparing the two lowest dilutions. Subsequent analyses should be carried out at the dilution giving the best results.

8.5 Choice of electrolyte solution

8.5.1 General

An electrolyte solution should be selected in which the sample is stable. The electrolyte solution should not dissolve, flocculate, react, or once a good dispersion is achieved, not change the state of dispersion of the sample in the measurement time, typically up to five minutes. Particles insoluble in water can be analysed in a variety of aqueous electrolyte solutions. Particles soluble in water can often be analysed in methanol or in isopropanol. See Reference [11] for recommended electrolyte solutions. When using small apertures (D < 50 µm) or large apertures (D > 400 µm), special care shall be taken due to their particular characteristics.

8.5.2 Special considerations for small apertures (D < 50 µm)

Where possible, the electrolyte solution should consist of an aqueous 4 % sodium chloride solution or one of equivalent conductivity. It should be membrane filtered twice at 0,2 µm or 0,05 µm for 10 µm apertures.

8.5.3 Special considerations for large apertures (D > 400 µm)

To prevent turbulence that can cause noise signals due to fast flow through the aperture, the viscosity of the electrolyte solution may be increased by the addition of glucose or glycerol; 10 % glycerol is recommended for 560 µm and 400 µm apertures, and 30 % glycerol for the 2 000 µm and 1 000 µm apertures.

8.6 Preparation of electrolyte solution

An electrolyte solution should be well filtered with a membrane filter for which the pore size is less than the diameter of the smallest particle measured, as it is essential that its background count should be as low as practicable. It should be noted that quoted values for filters are not absolute. Usually a mean pore size is given. The width of the distribution of pores around this mean varies depending on filter type and manufacturer. This will affect the choice of filter size used. All glassware and apparatus used should be pre-rinsed with filtered electrolyte solution or other suitable liquids. Background counts should not exceed the values given in Table 2 or yield a total equivalent volume in excess of 0,1 % of the total volume of particles subsequently measured in the analysis volume.

Table 2 — Counts for background for typical aperture diameters

Aperture diameter D µm	Analysis volume[a] V_m ml	Background counts[b]
1 000	2	2
560	2	10
400	2	25
280	2	75
200	2	200
140	2	600
100	0,5	400
70	0,5	1 200
50	0,05	300
30	0,05	1 500
20	0,05	5 000

[a] For other analysis volumes, use pro rata values.

[b] Suggested maximum counts.

8.7 Recommended sampling, sample splitting, sample preparation and dispersion

8.7.1 General

See ISO 14488 [18] for guidance on the sampling and sample-splitting procedure. Select an appropriate dispersant and a dispersion method (ISO 14487 [19] and Reference [11] provide guidance in this area). The expertise of the laboratory performing the analysis with respect to the sample under test may also be utilized.

Ultrasonic baths and probes are recommended for dispersion of materials. The time taken to disperse will be sample dependent. It is suggested that to optimize this that the sample be placed in the bath or probe and regular samples be taken over a time period of several minutes, every 30 s would be a suitable default. The size distribution over time can be monitored and a time point can be selected at which full dispersion has been achieved.

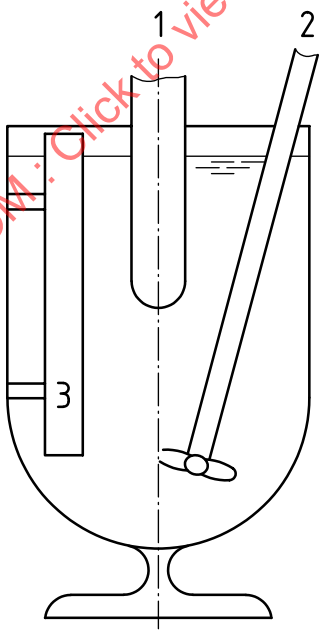
NOTE The use of high energy ultrasonic baths and probes, blenders and mixers can cause both agglomeration and fracture of particles.

8.7.2 Method 1: Using a paste

The sample should be subdivided to about 0,2 cm^3. If the sample is in the form of a powder, it should be worked and kneaded gently with a spatula and a few drops of suitable dispersant to break down agglomerates. Transfer a mass of about 20 mg to 50 mg of the paste to a round-bottomed beaker and add a few drops of electrolyte solution and thin it with dispersant. Almost fill the beaker with electrolyte solution and place it in an ultrasonic bath with suitable power and frequency for the time determined as per 8.7.1, stirring occasionally. A stopwatch is recommended for a reproducible dispersion technique. One suitable design of beaker of 400 ml capacity with a baffle is shown in Figure 3.

8.7.3 Method 2: Alternative method applicable to low-density particles of less than 50 µm

In this case, the density of the material will be close to the density of the electrolyte. Subdivide the sample into portions of about 1 g. Mix a portion with the dispersant and add it to the electrolyte solution. Then place the beaker (see Figure 3) containing the suspension in an ultrasonic bath for the time determined in 8.7.1. After stirring this stock suspension well, withdraw 5 ml using a pipette and add to approximately 400 ml of electrolyte solution. Place in the ultrasonic bath for a further 15 s. When using this method, it is important that at least two samples are withdrawn from the stock suspension and analysed to ensure repeatability of the aliquot sampling and the analysis.



Key

1 aperture tube
2 stirrer
3 baffle

Figure 3 — Example of a beaker with baffle and stirrer

8.7.4 Suspensions and emulsions

Suspensions and emulsions should be diluted by addition of smaller volumes of diluent to the emulsion, not by addition of the emulsion to a larger volume of diluent. Dilution should be performed stepwise with mixing performed at each step. To avoid "dilution shock", oil-in-water emulsions may be initially diluted with distilled or de-ionized water. If the suspension or emulsion is electrostatically stabilized the amount of electrolyte used should be minimised. The choice of diluent is important. If it needs to be diluted it should be diluted with an appropriate buffer which is inert to the particles and the act of dilution should not change the conductivity of the sample (if it does the system will require recalibration). Any diluent should also ideally be particle free and certainly at the very least should be prefiltered to remove any particles that could be measured at the given aperture size, i.e if a 20 µm aperture is being used, then the diluent should first be filtered at approximately 3 µm prior (0,15 *D*) prior to use.

8.7.5 Verification of the dispersion

A small sample of the dispersion may be placed on a microscope slide and used to verify the degree of dispersion and to estimate the size range of the particles using an optical microscope. If the observed particles are particularly acicular, there is potential for aperture blockage and incomplete measurement. For non-spheroidal material the largest diameter should be less than the aperture diameter (so it will not block the aperture if it passes end on). If an acicular particle with an equivalent spherical diameter of 0,02 *D* is considered as the smallest measurable particle, this gives a maximum length to breadth ratio of 400.

8.8 Choice of aperture(s) and analysis volume(s)

From the microscope examination (see 8.7.5), estimate the diameter of the largest particles present. Choose an aperture for the size analysis such that the diameter of the largest particles to be analysed is less than approximately 60 % of the diameter of the aperture, selected to reduce the possibility of blocking the aperture. For particles that are spherical or nearly spherical, an aperture such that the diameter of the largest particles is less than 80 % of the diameter of the aperture may be chosen. If there is a considerable proportion of sample below the lower size limit of that aperture (1,5 % of its diameter), a second and possibly a third smaller aperture will be needed (see Annex F).

Select a suitable analysis volume with reference to Table 1 or select a suitable time of accumulation. It may be necessary to analyse a number of analysis volumes or to accumulate for a long time to obtain an acceptable precision, e.g. 50 000 particles will yield a precision on the median of the distribution (coefficient of variation) of 0,4 %. Counting fewer particles will reduce the precision, but this may be necessary when using the larger apertures (see Clause 7 and Annex F).

8.9 Clearing an aperture blockage

Apertures below 100 µm in diameter may become blocked with extraneous particles, particularly if care is not exercised in the clean handling, careful filtration and thorough rinsing of beakers and associated equipment. A blockage or a partial blockage may be seen by means of the viewing optics, if provided with the analyser. A blockage may also be indicated by measuring the flow time through the aperture or by measuring the electrical resistance of the aperture. A blockage will cause a longer flow time and a higher resistance. A blockage can also be revealed by an examination of the particle pulse train, which is recorded with some instruments. A blockage will cause a clearly visible shift in the pulse train. In some instruments, there are means to automatically detect and remove blockages. Blockages can also be removed by one of the following techniques.

a) Back flushing: Reversing the flow through the aperture may be sufficient to clear a blockage.

b) Boiling: It is possible to use the heating effect of the current to boil the blockage out. This is done by using a high aperture current.

c) Brushing: It is often possible to brush the particles off the aperture by using a small high-quality soft-hair brush with the hair cut short. Care should be taken not to damage the aperture.

d) Air pressure.

e) Ultrasonic cleaning: With the aperture tube filled with electrolyte solution, the end is dipped into a low-power ultrasonic bath for about 1 s. Repeat this operation as necessary. This method is very effective but extreme care should be taken as it is possible to damage the aperture. **CAUTION — This method should not be used for apertures of 50 µm or less as can cause severe damage to the aperture.**

8.10 Stability of dispersion

The stability of the dispersion of particles can vary during the analysis time. Make a full-size distribution analysis as soon as possible after dispersion; then stir the dispersion for 5 min to 10 min and then reanalyse. When using a stirrer, adjust for maximum effect without creating a vortex that entrains bubbles. Cumulative counts are recorded at size levels close to 30 % and 5 % of the aperture diameter (denoted as x_{max} and x_{min} respectively). Changes in the counts greater than those expected from statistics will indicate that the dispersion is not stable (see Clause 7 and Annex F). Additional verification of stability can also be performed in instruments that record raw pulse data. Inhomogeneity across the pulse train during the time of analysis may indicate a change in the stability of dispersion. Table 3 details some possible causes. ISO/TR 13097 [20] provides practical guidelines for the assessment of dispersion stability and is recommended reading in this area.

Table 3 — Examples of suspected phenomena in dispersion

Change in count at		Suggests
x_{max}	x_{min}	
No change	No change	Stable dispersion
Increase	Increase	Crystallization, precipitation
Decrease	Decrease	Dissolution
Decrease	Increase	Size reduction, deflocculation
Increase	Decrease	Flocculation, agglomeration
Decrease	No change	Settling of large particles

8.11 Calibration

8.11.1 General

Electrical sensing zone instruments are typically calibrated using polymer latex microspheres of known size and narrow size distribution.

Those instruments which use the “constant current” approach should not require recalibration if the electrolyte conductivity is changed. Instruments using the “constant voltage” approach will require re-calibration for each electrolyte system to be used.

Another method, which is an absolute method, is the mass integration method (see Annex D). Here the weighed mass is compared to the mass found as determined by the instrument [12],[13]. This calibration method is directly traceable and there is no assumption made about the shape, porosity or electric conductivity of the particles.

Special care shall be taken for porous particles. Such particles may have an interconnected pore system which, at least partly, is being filled with electrolyte solution during the sample preparation procedure. This electrolyte solution will, to a certain extent, not contribute to the impedance change in the sensing zone when the particle passes through it. Therefore, a porous particle generates a pulse with lower amplitude than a solid particle of equivalent envelope volume. The difference is not negligible; for some porous materials the size can be as little as half that of the envelope size. For the calibration for the measurement of porous particles, see Annex E.

8.11.2 Calibration procedure — microsphere calibration

Microspheres with narrow size distribution with a single mode, characterized by a variety of other methods, are available. They should be characterized traceably to the metre, either by direct calibration or by comparison with a certified reference material (CRM) that itself has certified dimensions traceable to the metre obtained from a national metrology institute or another competent reference material producer. The calibration method used depends on the assayed size parameter of the microspheres and the analyser used (contact the instrument manufacturers for details). One method is to obtain a histogram (frequency) plot of the number of particles against channels of equal width (on a linear scale). The size at the centre of the channel with the greatest number of particles corresponds closely to the modal size of the calibration material if the distribution is symmetrical. If the distribution is not symmetrical, the fractional channel position is calculated from the counts in channels on either side of the central channel. The calibration factor is the ratio of the modal size of the calibration material to the size reported on the instrument. Modern instruments provide an "automated" calibration/verification standard operating method/routine.

Calibration should be checked on a regular basis to ensure that the change in the calibration constant is less than 1,0 %, or every time an aperture size or an electrolyte solution is changed. See Annex E for a method for calibration of frequently used apertures.

No certified particle number concentration standards exist at the time of publication of this document and the development of such concentration reference materials remains one of the challenges for achieving traceability in the concentration measurements. Most accurate concentration results are obtained when using a multi-point calibration procedure where particle rates for sample and calibration are measured at three or more pressures. Current standards are established through a combination of accurate determination of the size distribution of a traceable standard and gravimetry.

9 Analysis

Most powders have a particle size range that is sufficiently narrow for a satisfactory analysis to be carried out using one aperture. If an appreciable amount of relatively small particles are suspected to be present in the sample, their presence in the sample, their presence can be checked by executing a mass balance (see Annex D). Where the size range of a powder is too wide for a single aperture, two or more apertures should be used. If over 1,5 % by volume of the particles fall in the smallest size interval, it is advisable to use the multiple aperture method (see Annex E).

When the particles are dispersed satisfactorily, following the foregoing procedures [19], the analysis can begin. Select the analysis volume, or the number of repeat measurements of the analysis volume, or the time for accumulation, or the total number of particles to be measured, in such a way that a particle size distribution with sufficient precision is obtained (see Clause 7 and Annex F). Counting fewer particles will reduce the precision, but this may be necessary when using the larger aperture tubes. It is advisable that at least three, and preferably five, replicates be measured. The count numbers obtained are subject to errors in relation to Poisson statistics.

To ensure that the sample subdivision has been carried out well, the whole procedure should be repeated with at least one other, but preferably more, sample(s) from the stock suspension or from the dry powder subdivision. Report all the measured data on a suitable data sheet.

10 Calculation of results

Modern instruments measure the volume and the number of particles within various size channels directly, so no data conversion is needed. Some analysers count the number of particles above, or between, variable equivalent-volume particle diameters, and therefore conversion of data to volume percentage may be required. In the event of requiring number data to be converted to, and presented as, volume data, it is usual for the method of Simpson's rule [14] to be used. Since the volume of each particle is measured, the numbers of particles within a size interval (size channel) can be multiplied by the arithmetic mean volume of the channel in order to present the total particle volume within the channel. In this way, the total volume of all particles within all size channels can be calculated, and the

percentage by volume size distribution calculated. For the calculation to be reasonably accurate, the size interval should be narrow, i.e. a large number of channels should be counted. For a more accurate method and the calculation of moments of the distribution, see ISO 9276-2. Modern analysers perform the calculation automatically. The volume-percentage distribution so calculated is identical to the mass (or weight) distribution if all the particles have the same specific gravity (immersed density). It is also possible to calculate and display the surface area of the particles and thus total surface area by using the size and number information obtained. Such a calculation will be based on the assumption of spherical, non-porous particles.

11 Instrument qualification

11.1 General

The instrument is qualified through the verification of the linearity of the aperture/amplifier system (see 8.2), verification of the linearity of the counting system (see 8.3) and the verification of the calibration constant (see Annex C).

11.2 Report

It is essential that the whole qualification procedure and all data be reported in full detail on a suitable data sheet. The test report should contain as a minimum the following information:

a) identification of test specimen;

b) operator name;

c) complete description of method used for sample preparation;

d) date and time;

e) aperture size;

f) measurement conditions (current applied across two electrodes, amplified ratio calibration constant of diameter, etc.);

g) particle size distribution;

h) counts number of channel;

i) coincidence ratio (coincidence counts number of sample or sample concentration);

j) total number of particles;

k) concentration of sample.

Usable results should be reported in tables and graphs in accordance with ISO 9276-1 and ISO 9276-2.

Annex A
(informative)

Derivation of maximum count number to limit coincidence

The probability of coincidence by particles in the sensing zone may be described by a Poisson distribution:

$$P(k) = e^{-\alpha} \frac{\alpha^k}{k!} \quad \text{(A.1)}$$

where

k is the actual number of events per segment;

$k!$ is the factorial of k (= $k \cdot (k-1) \cdot (k-2) \cdot (k-3)$, where $k! = 1$ for $k = 0$ and $k = 1$...);

α is the average number of events per segment;

event = particle passage;

segment = sensing volume (V_{sens}).

5 % coincidence means that the number of coincidence counts is 0,05 × observed number of counts:

$$\frac{P(k>1)}{P(k=1)+P(k>1)} = \frac{\text{coincidence counts}}{\text{observed counts}} = 0{,}05 \quad \text{(A.2)}$$

It can be calculated that 5 % coincidence occurs for $\alpha = 0{,}1$:

$$P(k = 0) = 1/e^{0{,}1} = 0{,}905 \quad \text{(A.3)}$$

and

$$P(k = 1) = 0{,}1/e^{0{,}1} = 0{,}090 \quad \text{(A.4)}$$

thus:

$$P(k > 1) = 1 - (P(k = 0) + P(k = 1)) = 1 - 0{,}905 - 0{,}090 = 0{,}005 \quad \text{(A.5)}$$

Both the fact that the average number of events per segment α = 0,1 and the results of Formulae (A.3) to (A.6) show that the maximum particle concentration ($N_{5\,\%}$) for this 5 % coincidence is about 1 particle per 10 sensing volumes.

For cylindrical apertures, the sensing volume can be estimated from the diameter D and length L of the aperture. Typically, the sensing volume extends somewhat outside the aperture, the degree of which depends upon the aperture length. Lines et al. have indicated a factor 2,5 extension for one type of aperture ($L/D = 0{,}75$), Wynn et al. mention a factor 1,06 for another type of 60 µm aperture ($L/D = 4{,}8$).

While assuming that this factor 2,5 holds for all apertures having L/D = 1 and that longer apertures have a proportionally smaller factor, the sensing volume for all aperture volumes V_{ap} is:

$$V_{sens} = 2{,}5 \cdot V_{ap}, \text{ or } 2{,}5 \cdot ¼\pi \cdot D^3 \text{ fl, or about } 2 \cdot 10^{-12} \cdot D^3 \text{ ml} \quad \text{(A.6)}$$

One particle per 10 sensing volumes, as required for 5 % coincidence, means a particle concentration $N_{5\,\%}$ of:

$$N_{5\%} = 0{,}1/2 \cdot 10^{-12} \cdot D^3 \text{ ml, or about } 5 \cdot 10^{10}/D^3 \text{ particles per ml} \quad \text{(A.7)}$$

or

$$N_{5\%} = 5 \cdot 10^{10} \cdot V_a/D^3 \text{ particles per measured analysis volume } V_a \quad \text{(A.8)}$$

Annex B
(informative)

Fishbone diagram for method development

Figure B.1 illustrates the assessment of parameters which may determine how a particle sizing method is developed.

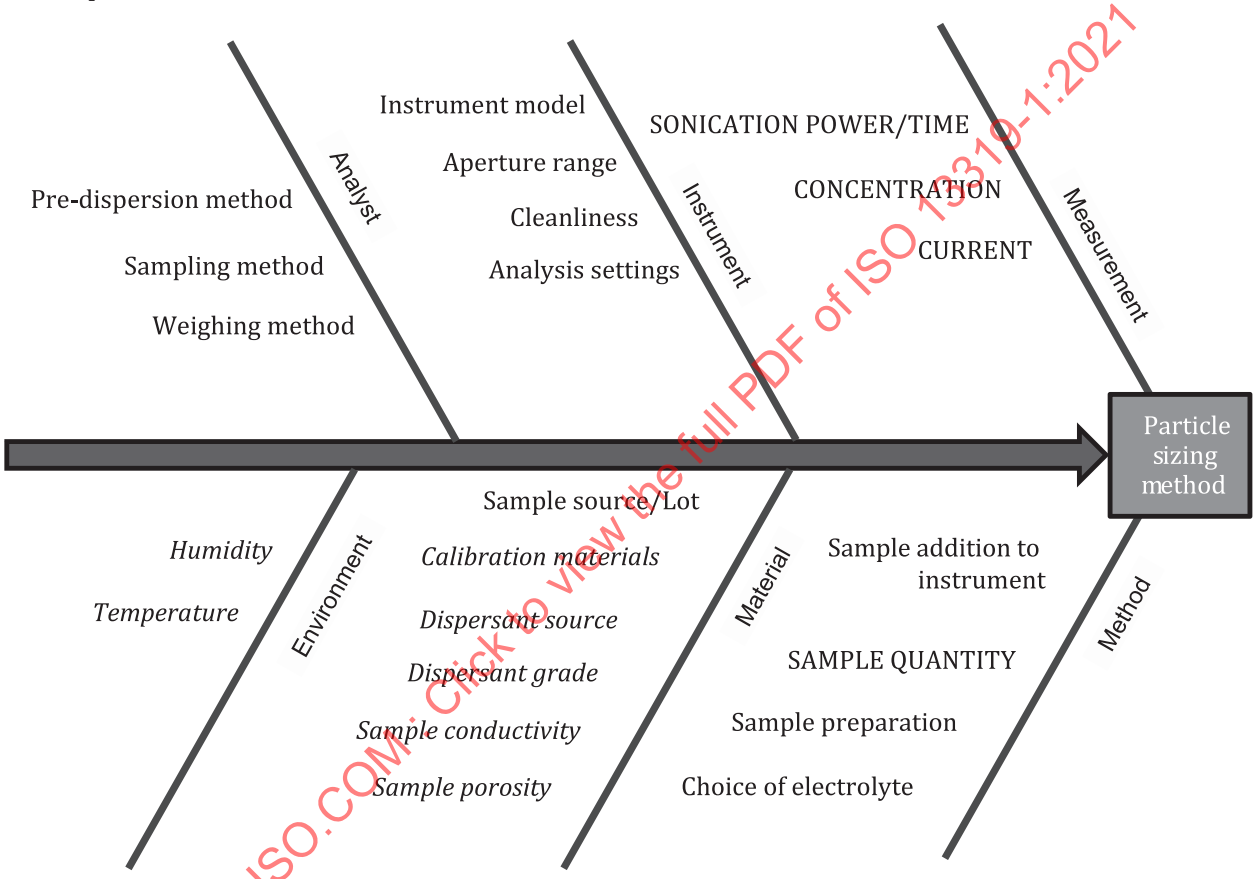


Figure B.1 — Fishbone diagram for method development

Once the user has a target method in mind, and also an idea of the desired method reproducibility, it is possible to start to define the method operable design region (MODR) for the method. To do this, an assessment of the main parameters which may impact the method is first needed. These can be grouped as follows:

a) **Analyst**: How the sample is obtained.

b) **Environment**: The conditions in the lab where the measurement will be made.

c) **Instrument**: The setup of the instrument and its basic functions (e.g. cleaning routines, aperture choice).

d **Material**: The source of sample and dispersant for the measurement, including the sourcing of any verification materials used to ensure the instrument is operating to specification. It will also take into account the conductivity and porosity of the sample.

e) **Measurement**: Parameters associated with the measurement and dispersion process.

f) **Method**: Parameters associated with the method set-up and selection process.

Within these, three groups of factors can be identified:

— **Noise factors** (italics in Figure B.1): Unintentional variations that if identified as potentially critical may require ruggedness testing to assess impact.

— **Control factors** (standard text in Figure B.1): Method factors which form part of the method definition and which can be specified at controllable unique levels in standard operating procedures (SOPs).

— **Experimental factors** (CAPITALS in Figure B.1): Method factors which form part of the method definition and which can be varied continuously and if potentially critical may require **robustness** experimentation to define the MODR.

For a method, the main parameters that need to be explored as part of a robustness study include:

— **Sonication power and time**: This will directly affect the state of dispersion of the sample.

— **Sample concentration**: Needs to be selected to ensure that a coincidence is minimised [21].

All the other parameters associated with the method can either be controlled to specific levels or are noise factors. However, they should not be forgotten in setting up the method.

NOTE 1 Robustness of an analytical method is the property that indicates the validity of a result with respect to minor changes in the various method parameters and hence its suitability for its defined purpose.

This is ascertained during the method development processes and determines the allowable (acceptable) limits for all critical parameters that affect measured values and provides information on a method's practicality.

NOTE 2 Ruggedness of an analytical method is the property that indicates the validity of a result with respect to inadvertent changes of known operational variables and in addition any variations (not discovered in intra-laboratory experiments) which can be revealed by interlaboratory studies. Such experiments are normally conducted on well-defined procedures following robustness experiment (or experiments) and provide information on a procedure's interlaboratory transferability.

Annex C
(informative)

Calibration and control of frequently used apertures

This annex describes a procedure applicable for the apertures that are used continually or very frequently. Twenty calibrations should be made consecutively using micro-spheres (see 8.11.2) over a short period of time (e.g. within a day or preferably during a week). Calculate the mean calibration constant $\overline{K}_\mathrm{d}$ and its standard deviation σ_{K_d} from this data. Plot the data in a Shewhart chart (as per ISO 7870-2:2013 [22]) with warning limits at

$$K_\mathrm{d} \pm 2\,\sigma_{\overline{K_\mathrm{d}}}$$

and action limits at

$$K_\mathrm{d} \pm 3\,\sigma_{\overline{K_\mathrm{d}}}$$

The mean calibration constant K_d is used in the subsequent analyses. The use of the mean ensures that random errors in the determination of the constant are reduced. Repeat the calibration immediately if the result falls between $\pm 2\,\sigma_{\overline{K_\mathrm{d}}}$ and $\pm 3\,\sigma_{\overline{K_\mathrm{d}}}$ (the warning area).

Take action in the form of cleaning or repair if two consecutive results are outside

$\pm 2\,\sigma_{\overline{K_\mathrm{d}}}$, or if one result is outside $\pm 3\,\sigma_{\overline{K_\mathrm{d}}}$ from the mean.

Annex D
(informative)

Mass integration method for calibration and mass balance

D.1 General

The mass integration method is generally believed to be close to an absolute method, provided that all particles in the suspension sample are properly measured, i.e. without any loss of counted particles by being too small to be detected in the aperture applied or by coincidence. Here, the ratio of input particle volume to output particle volume of the measurement is calculated, where input is derived from input mass, concentration and volume of suspension and effective particle density, and output is the total volume of particles counted. This method is directly traceable and there is no assumption made about the shape, porosity or electrical conductivity of the particles [12][13]. The method can be used both for calibration and for a mass balance. In a mass balance, it is possible to determine whether the measurement quality is adequate or significant errors are made. A first cause for errors is that only part of the particles is counted since the smallest particles are out of the range of the aperture. Also, the calibration constant may be inadequate due to errors coming from shape effects, porosity or electric conductivity of the particles. Furthermore, some instruments have a dead time in the pulse processing circuitry which can be significant and can correspond to an effective loss in the particles analysed by coincident travelling through the aperture. A loss will influence the result of a mass balance or a mass calibration procedure, therefore it is important to keep it low. The loss will depend on the concentration of particles counted. Therefore, a low particle concentration shall be used (e.g. with a coincidence risk lower than 5 %). For highest possible accuracy, coincidence-corrected counts should be used.

D.2 Calibration procedure

D.2.1 General

Mass calibration can be executed to compensate for any errors coming from shape effects, porosity or electric conductivity of the particles under investigation. A narrow size range of particles is applied that fits well in the aperture of the instrument. A known volume of dispersion is analysed, in which the particles are dispersed at a concentration that guarantees low coincidence.

D.2.2 Volume V_m of analysed suspension

In some instruments, the accuracy of V_m is guaranteed to better than 0,5 % for all settings, but in others only one metered volume may be guaranteed by the manufacturer. For these instruments, it is not sufficient to accept the nominal value of the analysis volumes. Using a suspension of particles, at a statistically valid count level (see Clause 7), five times measure the total coincidence-corrected particle count with the guaranteed volume. Switch to another analysis volume and obtain the total coincidence-corrected particle counts at least five times. The ratio of the total number of counts will be the ratio of the guaranteed volume to the selected volume. All counts should be recorded. The counts shall follow a chi-squared distribution, see Annex G.

D.2.3 Effective density of particles

Using a balance weighing to an accuracy of 0,1 mg, a relative-density bottle, pipettes and the usual particle-dispersion method, the effective density, ρ, of the material in the electrolyte solution to be used for the analysis is determined (see ISO 787-10). It is essential to test, in case of porous particles, that all open pores are filled with liquid in the procedure applied, since partial filling will lead to erroneous results. Closed pores will be measured as being part of the particle. Thus, their presence shall be accounted for in particle density.

D.2.4 Sample preparation

A narrow size-range fraction of the material under testing is prepared by sieving or a similar separation method. At least 99 % of the mass of the particles should lie within a size range of no more than 10:1, so that all can be measured using one aperture.

A suspension is prepared by dispersing a mass, m, of the powder in a volume, V_T, of the electrolyte solution and the dispersing agent.

D.2.5 Determination of the calibration factor

The calibration factor, K_d for the instrument is then determined by measuring very carefully the size distribution using an accurately known analysis volume, V_m, ensuring that the aperture is absolutely clear. A number of analysis volumes may be counted, or the analysis time may be long so that many particles are measured (e.g. 50 000 particles yields a coefficient of variation of 0,4 %). Fewer particles may be measured but this will lower the precision of the measurement. The particle concentration used should be less than 5 % coincidence limit. The coincidence-corrected counts shall be recorded and used in the calculations.

K_d is calculated from Formula (D.1):

$$K_d = K_{d,a}\left(\frac{V_m m}{V_T \rho \sum_{i=1}^{n} \Delta N_i \overline{V_i}}\right)^{1/3} \tag{D.1}$$

where

$$\overline{V_i} = \frac{\frac{\pi}{6}\left(d_L^3 + d_U^3\right)}{2} \tag{D.2}$$

and $K_{d,a}$ is an arbitrary value for the calibration constant to start the calculation of the calibration constant coming from mass calibration.

NOTE When one chooses the prior micro-sphere calibration constant for $K_{d,a}$ and one finds as a result the same value for K_d, which proves that the calibration constant is still valid and no effects are present coming from shape, porosity or electrical conductivity of the particles.

D.2.6 Secondary calibration

In general, calibration of the aperture by a micro-sphere suspension is recommended. It is recommended to use this mass-integration calibration procedure only in case of doubt for specific, e.g. porous products. In such cases, the two calibration constants can be compared and, if necessary, the typical microsphere calibration constant adjusted. It is not necessary to perform the full mass-integration calibration procedure for every analysis or for every aperture. Analyses reported using this secondary calibration should be identified.

D.2.7 Example of calibration by mass integration

See Table D.1.

Table D.1 — Example of calibration by mass integration

Diameter µm	$\overline{V}_i$ µm³	N_i	ΔN_i	$\Delta N_i \overline{V}_i$ µm³	$\sum \Delta N_i \overline{V}_i$ µm³
15–17	$2{,}169\ 79 \times 10^3$	487	487	$1{,}056\ 69 \times 10^6$	$1{,}056\ 69 \times 10^6$
17–19	$3{,}081\ 90 \times 10^3$	898	411	$1{,}266\ 66 \times 10^6$	$2{,}323\ 35 \times 10^6$
19–21	$4{,}220\ 21 \times 10^3$	1 301	403	$1{,}700\ 74 \times 10^6$	$4{,}024\ 09 \times 10^6$
21–23	$5{,}609\ 84 \times 10^3$	1 691	390	$2{,}187\ 84 \times 10^6$	$6{,}211\ 93 \times 10^6$
23–25	$7{,}275\ 93 \times 10^3$	2 190	499	$3{,}630\ 69 \times 10^6$	$9{,}842\ 62 \times 10^6$
25–27	$9{,}243\ 61 \times 10^3$	2 921	731	$6{,}757\ 08 \times 10^6$	$1{,}659\ 97 \times 10^7$
27–29	$1{,}153\ 80 \times 10^4$	4 175	1 254	$1{,}446\ 87 \times 10^7$	$3{,}106\ 84 \times 10^7$
29–31	$1{,}418\ 43 \times 10^4$	6 678	2 503	$3{,}550\ 33 \times 10^7$	$6{,}657\ 17 \times 10^7$
31–33	$1{,}720\ 76 \times 10^4$	11 602	4 924	$8{,}473\ 00 \times 10^7$	$1{,}513\ 02 \times 10^8$
33–35	$2{,}063\ 29 \times 10^4$	20 384	8 782	$1{,}811\ 98 \times 10^8$	$3{,}325\ 00 \times 10^8$
35–37	$2{,}448\ 56 \times 10^4$	31 583	11 199	$2{,}742\ 14 \times 10^8$	$6{,}067\ 14 \times 10^8$
37–39	$2{,}879\ 06 \times 10^4$	42 342	10 759	$3{,}097\ 58 \times 10^8$	$9{,}164\ 72 \times 10^8$
39–41	$3{,}357\ 32 \times 10^4$	50 083	7 741	$2{,}598\ 90 \times 10^8$	$1{,}176\ 36 \times 10^9$
41–43	$3{,}885\ 84 \times 10^4$	54 548	4 465	$1{,}735\ 03 \times 10^8$	$1{,}349\ 86 \times 10^9$
43–45	$4{,}467\ 14 \times 10^4$	56 803	2 255	$1{,}007\ 34 \times 10^8$	$1{,}450\ 59 \times 10^9$
45–47	$5{,}103\ 73 \times 10^4$	57 840	1 037	$5{,}292\ 57 \times 10^7$	$1{,}503\ 52 \times 10^9$
47–49	$5{,}798\ 12 \times 10^4$	58 369	529	$3{,}067\ 21 \times 10^7$	$1{,}534\ 19 \times 10^9$
49–51	$6{,}552\ 84 \times 10^4$	58 659	290	$1{,}900\ 32 \times 10^7$	$1{,}553\ 19 \times 10^9$
51–53	$7{,}370\ 39 \times 10^4$	58 804	145	$1{,}068\ 71 \times 10^7$	$1{,}563\ 88 \times 10^9$
53–55	$8{,}253\ 28 \times 10^4$	58 894	90	$7{,}427\ 95 \times 10^6$	$1{,}571\ 37 \times 10^9$
55–57	$9{,}204\ 03 \times 10^4$	58 936	42	$3{,}865\ 69 \times 10^6$	$1{,}575\ 24 \times 10^9$
57–59	$1{,}022\ 52 \times 10^5$	58 963	27	$2{,}760\ 80 \times 10^6$	$1{,}578\ 00 \times 10^9$
59–61	$1{,}131\ 92 \times 10^5$	58 976	13	$1{,}471\ 50 \times 10^6$	$1{,}579\ 47 \times 10^9$
61–63	$1{,}248\ 86 \times 10^5$	58 987	11	$1{,}373\ 75 \times 10^6$	$1{,}580\ 84 \times 10^9$
63–65	$1{,}373\ 59 \times 10^5$	58 993	6	$8{,}241\ 54 \times 10^5$	$1{,}581\ 66 \times 10^9$
65–67	$1{,}506\ 36 \times 10^5$	58 993	0	$0{,}000\ 00 \times 10^5$	$1{,}581\ 66 \times 10^9$

$K_{d,a} = 294{,}1$

$V_m = 100{,}000$ ml

$m = 25{,}2$ mg $= 25{,}2 \times 10^{-3}$ g

$V_T = 333{,}695\ 0$ ml

$\rho = 2{,}23$ g/ml

$$\sum \Delta N_i \overline{V}_i = 1{,}581\ 66 \times 10^9\ (\mu m)^3 = 1{,}581\ 66 \times 10^9 \times 10^{-12}\ ml$$

$$K_d = 294{,}1\sqrt[3]{\frac{100{,}00 \times 25{,}2 \times 10^{-3}}{333{,}6950 \times 2{,}23 \times 1{,}581\ 66 \times 10^{9} \times 10^{-12}}}$$

$$K_d = 379{,}1$$

The mean particle volume of a size interval $\Delta N_i \overline{V}_i$ and the total particle volume $\sum_{i=1}^{n} \Delta N_i \overline{V}_i$ may be calculated by the computer software provided with the particle size analyser. For precise information about the specific software, the instrument manufacturer or the instrument operation manual should be consulted.

D.3 Mass balance

D.3.1 General

The mass balance compares the volume of the particles measured by the instrument, multiplied with the effective particle density, with the powder sample mass. The ratio of these two values gives the fraction of powder that is accounted for in the measurement. This ratio gives guidance as to whether the smallest particles are accounted for or any other significant errors have occurred. Typically, a mass balance of 100 % ± 5 % can be met. See also D.1.

D.3.2 Sample preparation

A suspension is prepared by dispersing a mass, m, of the powder in a volume, V_T, of the electrolyte solution and the dispersing agent. Weigh the sample and the electrolyte solution with highest possible accuracy.

D.3.3 Procedure

When the particles are dispersed satisfactorily, the analysis of a known volume V_m can begin. Measure very carefully, on a linear scale, the size distribution ensuring that the aperture is absolutely clear. For the calculation to be reasonably accurate, the size interval should be narrow (i.e. a large number of channels should be counted, the number of channels across the particle size distribution should be as large as possible as narrow size intervals allow better evaluation of the mean size). The coincidence-corrected counts shall be recorded and used in the calculations.

D.3.4 Calculations

The mass measured by the instrument may be calculated by the Formula (D.3)

$$M_m = \frac{\sum \Delta N_i \overline{V}_i (\rho \cdot V_T)}{V_m} \tag{D.3}$$

The particle mass accounted for, in percent, may be calculated by Formula (D.4)

$$M_b = M_m / m \times 100 \tag{D.4}$$

where

M_m is the mass of particles measured by the instrument;

M_b is the percentage of particles accounted for in a measurement in comparison to input particle mass;

m is the mass, of the powder dispersed in a volume, V_T.

D.3.5 Results

The ratio of the mass of the particles measured by the instrument and the true particle concentration, as determined by weighing the powder sample dispersed in the weighed volume of electrolyte, should deviate from unity by no more than 5 %. A value deviating by more than 5 % indicates that not all particles can be measured using one aperture or that errors are made due to effects coming from sampling, the state of dispersion, shape, porosity or electrical conductivity of the particles. When it can be assumed that only part of the particles is measured, then the two (or more) aperture technique (see Annex F), should be used, if possible, to achieve highest possible accuracy.

D.3.6 Report

It is essential that the whole mass integration procedure and all data be reported on a suitable data sheet.

D.3.7 Theoretical example of mass balance

See Table D.2.

Table D.2 — Example of mass balance

Diameter µm	$\overline{V}_i$ µm³	N_i	ΔN_i	$\Delta N_i \overline{V}_i$ µm³	$\sum \Delta N_i \overline{V}_i$ µm³
19,33 to 21,91	$4{,}644\,45 \times 10^3$	487	487	$2{,}261\,85 \times 10^6$	$2{,}261\,85 \times 10^6$
21,91 to 24,49	$6{,}598\,91 \times 10^3$	898	411	$2{,}712\,15 \times 10^6$	$4{,}974\,00 \times 10^6$
24,49 to 27,07	$9{,}038\,52 \times 10^3$	1 301	403	$3{,}642\,52 \times 10^6$	$8{,}616\,52 \times 10^6$
27,07 to 29,65	$1{,}201\,72 \times 10^4$	1 691	390	$4{,}686\,72 \times 10^6$	$1{,}330\,32 \times 10^7$
29,65 to 32,23	$1{,}558\,90 \times 10^4$	2 190	499	$7{,}778\,92 \times 10^6$	$2{,}108\,22 \times 10^7$
32,23 to 34,80	$1{,}979\,83 \times 10^4$	2 921	731	$1{,}447\,25 \times 10^7$	$3{,}555\,47 \times 10^7$
34,80 to 37,38	$2{,}470\,70 \times 10^4$	4 175	1254	$3{,}098\,26 \times 10^7$	$6{,}653\,73 \times 10^7$
37,38 to 39,96	$3{,}037\,87 \times 10^4$	6 678	2503	$7{,}603\,78 \times 10^7$	$1{,}425\,75 \times 10^8$
39,96 to 42,54	$3{,}685\,89 \times 10^4$	11 602	4924	$1{,}814\,93 \times 10^8$	$3{,}240\,68 \times 10^8$
42,54 to 45,12	$4{,}420\,18 \times 10^4$	20 384	8782	$3{,}881\,80 \times 10^8$	$7{,}122\,48 \times 10^8$
45,12 to 47,69	$5{,}244\,34 \times 10^4$	31 583	11 199	$5{,}873\,14 \times 10^8$	$1{,}299\,56 \times 10^9$
47,69 to 50,27	$6{,}165\,35 \times 10^4$	42 342	10 759	$6{,}633\,30 \times 10^8$	$1{,}962\,89 \times 10^9$
50,27 to 52,85	$7{,}190\,39 \times 10^4$	50 083	7 741	$5{,}566\,08 \times 10^8$	$2{,}519\,50 \times 10^9$
52,85 to 55,43	$8{,}323\,24 \times 10^4$	54 548	4 465	$3{,}716\,33 \times 10^8$	$2{,}891\,13 \times 10^9$
55,43 to 58,01	$9{,}569\,31 \times 10^4$	56 803	2 255	$2{,}157\,88 \times 10^8$	$3{,}106\,92 \times 10^9$
58,01 to 60,58	$1{,}093\,11 \times 10^5$	57 840	1 037	$1{,}133\,56 \times 10^8$	$3{,}220\,28 \times 10^9$
60,58 to 63,16	$1{,}241\,67 \times 10^5$	58 369	529	$6{,}568\,42 \times 10^7$	$3{,}285\,96 \times 10^9$
63,16 to 65,74	$1{,}403\,42 \times 10^5$	58 659	290	$4{,}069\,93 \times 10^7$	$3{,}326\,61 \times 10^9$
65,74 to 68,32	$1{,}578\,66 \times 10^5$	58 804	145	$2{,}289\,06 \times 10^7$	$3{,}349\,55 \times 10^9$
68,32 to 70,90	$1{,}767\,91 \times 10^5$	58 894	90	$1{,}591\,12 \times 10^7$	$3{,}365\,46 \times 10^9$
70,90 to 73,47	$1{,}971\,30 \times 10^5$	58 936	42	$8{,}279\,45 \times 10^6$	$3{,}373\,74 \times 10^9$
73,47 to 76,05	$2{,}189\,75 \times 10^5$	58 963	27	$5{,}912\,32 \times 10^6$	$3{,}379\,65 \times 10^9$
76,05 to 78,63	$2{,}424\,23 \times 10^5$	58 976	13	$3{,}151\,50 \times 10^6$	$3{,}382\,81 \times 10^9$
78,63 to 81,21	$2{,}674\,88 \times 10^5$	58 987	11	$2{,}942\,37 \times 10^6$	$3{,}386\,08 \times 10^9$
81,21 to 83,79	$2{,}942\,25 \times 10^5$	58 993	6	$1{,}765\,35 \times 10^6$	$3{,}387\,84 \times 10^9$
83,79 to 86,37	$3{,}226\,87 \times 10^5$	58 993	0	0,000 00	$3{,}387\,84 \times 10^9$

V_m = 100,00 ml

m = 25,2 mg = $25{,}2 \times 10^{-3}$ g

V_T = 333,695 0 ml

ρ = 2,23 g/ml

$$\sum \Delta N_i \overline{V}_i = 3{,}387\ 84 \times 10^9 \left(\mu m^3\right) = 3{,}387\ 84 \times 10^9 \times 10^{-12}\ \text{ml}$$

Using Formulae (D.3) and (D.4) gives

$$M_m = \frac{3{,}387\ 84 \times 10^9 \times 10^{-12} \times 2{,}23 \times 333{,}695\ 0}{100{,}00}\ \text{g} = 0{,}025\ 21\ \text{g}$$

$$M_b = \frac{0{,}025\ 21}{25{,}2 \times 10^{-3}} \times 100 = 100{,}0$$

The recovery of particles is 100 % with the aperture in use.

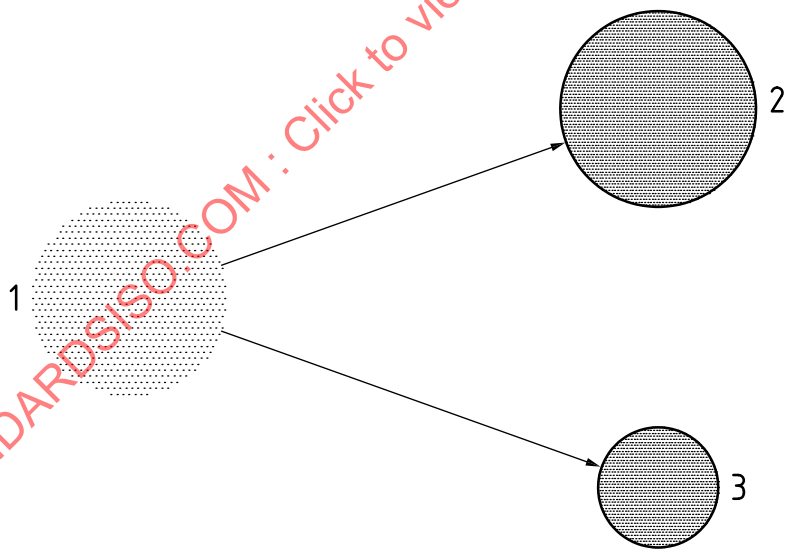
Annex E
(informative)

Calibration for the measurement of porous and conductive particles

E.1 General

Particles of conductive materials (e.g. metal particles) can be accurately measured by the electrical sensing zone method, provided that the voltage applied across the aperture does not prohibit the formation of a surface layer, the Helmholtz layer. This voltage may typically be 10 V to 15 V [4].

Porous particles have a pore structure which may be filled with electrolyte solution during the sample preparation procedure. This electrolyte solution will, at least to a certain extent, not contribute to the impedance change in the sensing zone of the aperture when the particle passes through it. Therefore, a porous particle generates a pulse with lower amplitude than a solid particle of equivalent envelope volume. If the calibration of the instrument is done with solid particles and no correction is carried out for the effect of the porosity, the measured size will be too small (see Figure E.1). A technique to solve this problem is to fill the pores either with an organic substance that is solid at room temperature or with a solvent that is immiscible with water [15],[16]. However, this technique cannot be used with porous particles made of natural polymers because they change size in organic solvents. Porous particles can be accurately measured after calibration of the measuring instrument using the mean size of a narrowly sized fraction of the material under investigation as described in E.3.



Key

1 porous particle
2 reported size with correction for the porosity (envelope size)
3 reported size without correction for the porosity

Figure E.1 — Diagram illustrating the response of a porous particle

E.2 Particles of conductive materials

To obtain acceptable results for conductive particles, such as metal particles, a distribution is obtained under normal conditions. The analysis is then repeated using half the current and twice the gain. The distributions should be the same. If they are not, the procedure should be repeated using an even lower current. Some metal particles are highly conductive (e.g. copper, silver and platinum). These particles do not easily form surface layers, but they can be correctly sized if a very low voltage is applied and the barrier increased by adding a 0,5 % solution of cetrimonium bromide.

E.3 Porous particles

E.3.1 General

In order to compensate for the effects of the porosity, the scaling of pulse height is done with the material under test. A narrow size-range fraction of that material is prepared by sieving or a similar separation method. The mean diameter is then determined by computer-aided microscopy, image analysis, or by measurements on photographs. Finally, the material is analysed by the electrical sensing zone (ESZ) instrument and the known mean diameter is used for calibration of the instrument [9],[10].

E.3.2 Sample preparation

In the case of sieving, the sieves should have openings as close as possible to the modal diameter of the sample (e.g. 5 µm to 10 µm on each side). Wet sieving with electro-formed sieves is preferred. The sieving fluid may be chosen according to the expertise of the laboratory to fit the actual material as well as possible. The size of porous materials may vary with the ion strength of the suspension medium. Therefore, the fraction shall be transferred to the electrolyte solution in which the size is to be expressed. The sample is then allowed to stand overnight or treated in an ultrasonic bath in order to substitute the liquid inside the particle for electrolyte solution.

E.3.3 Microscopy and ESZ measurements

An aliquot of the suspension of the sieved fraction is transferred to a microscope slide and covered with a cover glass. Then photographs are taken, or the particle size is measured directly with an image analyser. It is important that the sample is not allowed to dry. At least 400 particles should be measured [9]. The number of particles to be measured can also be calculated using the procedure described in ISO 13322-1 [17]. The modal diameter of the number distribution should be the preferred size parameter for calibration, but the median diameter either in number or in volume distribution may also be used. Finally, the particle size is measured with the ESZ instrument which has been calibrated with microspheres.

E.4 Calculations for microscopy

E.4.1 General

The mean diameters of the microspheres and the sieved fraction are used to calculate a response factor which can be used for future calibrations.