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STANDARD

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**Particle size analysis — Photon correlation  
spectroscopy**

*Analyse granulométrique — Spectroscopie par corrélation de photons*



Reference number  
ISO 13321:1996(E)

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13321 was prepared by Technical Committee ISO/TC 24, *Sieves, sieving and other sizing methods*, Subcommittee SC 4, *Sizing by methods other than sieving*.

Annex A forms an integral part of this International Standard. Annexes B to F are for information only.

## Introduction

Particle sizing in the submicrometer size range is nowadays performed on a routine basis using photon correlation spectroscopy (PCS). The success of the technique is mainly based on the facts that it provides estimates of average particle size in measuring times of a few minutes and that user-friendly commercial equipment is available. Nevertheless, proper use of the instrument and interpretation of the results require certain precautions. Therefore there is a need for an International Standard for the determination of particle size by photon correlation spectroscopy, in order to provide a methodology that allows the users to obtain good interlaboratory agreement on accuracy and reproducibility.

Although PCS allows the determination of particle size distribution, this International Standard is limited to the description of size distribution by means of only two parameters: an average size and a polydispersity index, as obtained by so-called cumulants analysis (see annex A). This does not exclude more detailed information about particle size distributions being obtained. However, the reproducibility and reliability of the method of calculation for full distributions is, at the present state of the art, not good enough to include in an International Standard. Again, this does not exclude determination of acceptable size distributions in particular applications.

This International Standard recommends measurements at a single scattering angle of  $90^\circ$  using a He-Ne laser light source with a wavelength *in vacuo* of 632,8 nm. Since solid state laser sources operating at other wavelengths have become available and may be used in future instruments, this International Standard already includes recommendations for such instruments. Although the procedure given is limited to a single angle measurement, with some instruments additional measurements and valuable additional information can be obtained at other scattering angles or by simultaneous analysis of measurements performed at different angles.

This International Standard uses isotropic spherically shaped particles within a test procedure. Measurement of nonspherical and/or nonisotropic particles can be made by this technique where the size of such particles is reported by a spherical equivalence.

A list of suitable references for further reading is given in annex F.

# Particle size analysis — Photon correlation spectroscopy

## 1 Scope

This International Standard describes the application of photon correlation spectroscopy (PCS) to the measurement of an average particle size and a measure of the broadness of the size distribution of particles dispersed in liquids. It is applicable to particle sizes ranging from a few nanometres to about 1  $\mu\text{m}$ , or to the onset of sedimentation. In the data analysis procedure (see annexes A and C) it is assumed that the particles are isotropic and spherically shaped.

NOTE 1—The technique is also known or referred to under other names, e.g. quasi-elastic light scattering (QELS) and dynamic light scattering (DLS).

## 2 Definitions

For the purposes of this International Standard, the following definitions apply.

**2.1 average particle diameter,  $x_{\text{PCS}}$ :** Harmonic intensity-averaged particle diameter, as determined by equation (C.10) of annex C.

It is expressed in nanometres ( $10^{-9}$  m).

**2.2 polydispersity index, PI:** Dimensionless measure of the broadness of the size distribution, as determined by equation (C.9) of annex C.

**2.3 scattering volume,  $V$ :** Section of the incident laser beam viewed by the collecting or detector optics.

Typical order of magnitude is  $10^{-6}$   $\text{cm}^3$ .

## 3 Symbols

$B$  value of the intercept of the intensity autocorrelation function [see equation (C.6) of annex C];

$B_{\text{max}}$  maximum value of the intercept  $B$  for a given setting of the detection optics;

$c$  concentration of particulate material, in moles per litre;

$G_2(\tau)$  intensity autocorrelation function;

$n$  refractive index of the dispersion medium;

$N_V$  number of particles in scattering volume  $V$ ;

$\eta$  viscosity of the dispersion medium;

$\Gamma$  decay rate;

$\lambda_0$  laser wavelength *in vacuo* (632,8 nm for He-Ne laser);

$\phi$  particle volume fraction;

$\rho$  particle density;

$\theta$  scattering angle;

$\mu_2$  second cumulant.

## 4 Principle

A monochromatic and coherent laser light beam illuminates a representative sample for particle size analysis, dispersed at a suitable concentration in a liquid. The light scattered by the particles at an angle (typically  $90^\circ$ ) is recorded by a detector whose output is fed to a correlator. The decay of the autocorrelation function of the scattered intensity is interpreted in terms of average particle size and polydispersity index by the so-called cumulants method.

Annex C provides some theoretical background for particle sizing by PCS.

## 5 Apparatus

The main components of a typical photon correlation spectrometer are listed below.

NOTE 2 — Commercial or "home-made" instruments that meet the requirements of this International Standard may be used. There are several significant differences, both in hardware and software, not only between instruments from different manufacturers but also between different types from one manufacturer. The instrument specifications do not always give adequate information for proper assessment of its specific features. Therefore, annex D has been provided to recommend specifications for PCS instruments.

**5.1 Laser**, monochromatic, emitting light polarized with its electric field component perpendicular to the plane formed by the incident and detected rays (vertical polarization), e.g. a He-Ne laser capable of 2 mW to 5 mW power output.

**5.2 Sample holder**, allowing control and measurement of the temperature to within  $\pm 0,3$  °C.

**5.3 Primary beam stop**.

**5.4 Optics and detector**, to collect and digitize the radiation scattered by the sample at an angle of, e.g., 90°. If a polarization analyzer is included, it shall be positioned in the vertical position, i.e. with a maximum transmission for light polarized with its electrical field perpendicular to the plane of incident and scattered beams.

**5.5 Correlator**.

**5.6 Computation unit**.

## 6 Preliminary procedures

### 6.1 Instrument location

The instrument shall be placed in a clean environment, free from excessive electrical noise and mechanical vibration and out of direct sunlight. If organic liquids are used (e.g. as an index-matched liquid and/or as the suspension medium), there shall be due regard to local health and safety requirements, and the area shall be well ventilated. The instrument shall be placed on a rigid table or bench to avoid necessity for frequent realignment of the optical system.

NOTE 3 — Alternatively, it may incorporate a rigid optical bench internally.

**WARNING — PCS instruments are equipped with low or medium power lasers whose radiation can cause permanent eye damage. Never look into the direct path of the laser beam or its reflections. Do not use highly reflecting surfaces when the laser**

**beam is on. Observe local regulations for laser radiation safety.**

### 6.2 Sample preparation and inspection

**6.2.1** Samples shall consist of well-dispersed particles in a liquid medium. The dispersion liquid shall fulfil following requirements:

- it shall be transparent (non-absorbing) at the laser wavelength;
- it shall be compatible with the materials used in the instrument;
- it shall not dissolve, swell or coagulate the particulate material;
- it shall have a refractive index different from that of the particulate material;
- its refractive index and viscosity shall be known with an accuracy better than 0,5 %;
- it shall be well filtered.

Water is often used as a dispersion medium. The use of freshly distilled water (the still shall be built from quartz glassware) or of deionized and filtered (pore size 0,2  $\mu\text{m}$ ) water is recommended. Since long-range particle interaction may affect the results for strongly charge-stabilized dispersions, a trace of salt [c(NaCl) about  $10^{-3}$  mol/l] may be added to such samples to reduce the range of particle interaction.

Large fluctuations in recorded scattered time-averaged signals (count rate) on short time scales (e.g. 0,1 s intervals) with bursts of high count rates indicate the presence of contaminating dust. The appearance of sparkling centres in the beam also usually indicates a dusty sample. Such liquids shall be further cleaned (by filtration and/or distillation) before use.

Detailed recommendations for sample preparation are given in annex E.

The dispersion liquid alone shall give no (or very low) scattered signal when checked in the instrument for dust or contaminants.

**6.2.2** The concentration of particulate material shall be above some minimum level and shall not exceed a maximum level. The minimum level is determined by the following two requirements:

- The scattered intensity (count rate) of the sample containing the dispersed particles shall be at least 10 times higher than the signal scattered by the dispersion medium alone.
- The number  $N_V$  of particles in the scattering volume shall be at least about 1 000 (any number in the range 500-1 000 is acceptable).

NOTE 4 — This number can be estimated from the average PCS diameter  $x_{PCS}$ , from the particle volume fraction  $\phi$  and from the value  $V$  of the measuring volume by

$$N_V = 6\phi V / \pi x_{PCS}^3$$

A typical order of magnitude of  $V$  is  $10^{-6}$  cm<sup>3</sup> (its value can be found in the specifications provided by the instrument manufacturer). This equation relates to monosized materials only; for polydisperse samples the actual number of particles in the scattering volume may be much larger than predicted by this equation. If this larger number density compromises the single-scattering criteria 3) to 5), it may be that further dilution is necessary, leading to a requirement that either the coherence aperture in the receiver be increased or the incident laser beam made larger to increase the measurement volume. A subsequent reduction in measured intercept will then be incurred. This compromise is not permitted for the purposes of calibration and verification.

The maximum level is mainly determined by the condition that only single scattering shall be observed, i.e. no multiple scattering. The absence of significant contributions from multiple scattering shall be determined by the three following checks.

- 3) The samples shall not look opaque but shall look clear, or only slightly cloudy or turbid. This shall always be verified before putting the sample into the instrument.
- 4) The ratio of the measured intercept (see annex A for its determination) to its maximal value (see the specifications of the manufacturer or clause 8 for its determination) shall be at least 0,8.
- 5) If the instrument allows the laser beam passing through the sample to be viewed indirectly, a sharp parallel beam shall be observed; a spreading halo around the beam or any apparent absorbance shall not be present.

NOTE 5 — If possible, the following additional check is recommended. If a spectrophotometer is available, the optical density (O.D.) of the sample at the laser wavelength used for a 1-cm path length can be used as an indicator for multiple scattering; for O.D. values larger than 0,04, multiple scattering effects are to be expected.

In many applications a volume fraction ( $\phi$ ) of dispersed particulate material in the range  $10^{-5}$  to  $10^{-4}$  fulfils the requirements for particle sizes below about 500 nm. [In order to obtain the concentration range ( $c$ ) in mass of dispersed material per unit volume, multiply the volume fraction by the particle density  $\rho$  ( $c = \rho\phi$ ).] For polydisperse and/or larger particles, it may not be possible to find a concentration that satisfies requirements 2), 3) and 4) without either increasing the coherence aperture of the receiver or increasing the diameter of the incident laser beam in order to increase the measurement volume. If this is so, then the intercept values obtained may not meet the criterion set out in 4). For particle sizes above 1  $\mu$ m, the

requirements 2), 3) and 4) can only be fulfilled in exceptional cases.

## 7 Measurement procedure

The measurement procedure assumes a properly installed and aligned instrument and an operator familiar with the instrument manual.

**7.1** Switch the instrument on and allow it to warm up.

Typically about 0,5 h is required to stabilize the laser intensity and to bring the sample holder to the desired temperature.

**7.2** Check the dispersion medium and record the average count rate scattered by a blank sample of it.

**7.3** Place a sample of the dispersion (dispersion medium + particulate sample) in the instrument and allow temperature equilibrium to be established between sample and sample holder. The temperature shall be controlled and measured with an accuracy of 0,3 °C.

NOTE 6 — It can take about 10 min for the liquid in the measurement volume of a 1-cm measuring cell to equilibrate by thermal diffusion for a temperature change of only 3 °C. Errors in particle size determined from aqueous dispersions will be recorded at the rate of 2 % per °C if the temperature of the sample does not reach equilibrium.

A possible alternative method is to measure the room temperature and then set the instrument to control the sample holder temperature to within 0,3 °C of the room temperature. Samples can then equilibrate at room temperature and be measured immediately after insertion in the sample holder. Alternatively, samples can be equilibrated in a thermostatted bath whose temperature is within 0,3 °C of that of the instrument sample holder. In this case, remove the water from the cell before it contaminates the index-matching fluid.

**7.4** The following data shall be recorded: sample identification, laser wavelength and scattering angle if necessary, measuring temperature, refractive index and viscosity of the dispersion medium, particle concentration and any other relevant details.

**7.5** Perform a preliminary measurement on the dispersion to check that the particle concentration is not too low [less than about 1 000 particles in the scattering volume, see 6.2.2 check 2)] or too high [see 6.2.2, check 4)]. Check that the average scattered intensity (count rate) is in the range 5 kcounts/s to 1 000 kcounts/s (5 000 counts/s to 1 000 000 counts/s). If these criteria are not met, repeat a measurement on a sample with a different concen-

tration of particulate material, or use another laser power level if possible.

**7.6** For each sample, at least six duplicate measurements of at least 60 s duration shall be performed and results stored. For sample count rates below 20 000 counts/s (20 kcounts/s), the duration in seconds shall be equivalent to at least 1 200 000 counts divided by the count rate in counts per second, or 1 200 kilocounts divided by the count rate in kilocounts per second.

**7.7** Record the average particle diameter  $x_{PCS}$  and polydispersity index PI for each of the measurements. Calculate the mean:

$$\langle x \rangle = \frac{1}{N} \sum_{i=1}^N x_i$$

and the estimated standard deviation:

$$s_{N-1} = \frac{1}{N-1} \sqrt{\sum_{i=1}^N (x_i - \langle x \rangle)^2}$$

where  $N$  is the number of measurements  $x_i$  ( $i = 1 \dots N$ ) of  $x_{PCS}$  and of PI.

**7.8** If a systematic concentration dependence of average particle size is observed, the results of an extrapolation to infinite dilution (or the results obtained at the lowest acceptable concentration) shall be reported.

NOTE 7 — Although checks 3) to 5) of 6.2.2 will exclude biasing effects due to multiple scattering, particle interactions may, in particular for smaller particles ( $x_{PCS} \leq 100$  nm) at volume fractions above 0,01, bias the estimation of the average PCS diameter via equation (C.5) of annex C. Therefore for unknown dispersed systems it is recommended that steps in 7.5 to 7.7 are repeated for several concentrations in a range of a factor of at least 2.

**7.9** Check at the end of the measurement that no significant sedimentation has occurred in the sample. If sediment is found then either the sample may have aggregated and precipitated or the material may be fundamentally unsuitable for measurement by PCS.

## 8 Calibration and validation

Since particle size determination by PCS is an absolute method based upon first principles, calibration as such is unnecessary. However, verification of the instrument's performance should be performed with dispersions of particles of certified size after the first

installation of the instrument and at time intervals thereafter or in case of doubt.

Dispersions of polystyrene latex with narrow size distribution with average particle diameter as measured by PCS of about 100 nm shall be used. For such dispersions the measured average particle size shall be within 2 % of the stated size and the repeatability, defined as  $100s_{N-1}/\langle x \rangle$ , shall be better than 2 %; the polydispersity index shall be smaller than 0,1.

NOTE 8 — Several suppliers provide traceable latices. A polystyrene latex (catalogue No. 1963) with a certified diameter of 100,7 nm can be obtained from the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA. Annex E provides guidelines for the preparation of a suitable sample.

The maximal value of the intercept of the autocorrelation function can be determined as follows:

- select a collecting aperture for which a value of  $B_{\max}$  is available;
- determine the intercept  $B$  (see annex A) with dispersions of polystyrene latex (diameter about 100 nm) with at least two different concentrations meeting requirements 1), 2), 3) and 5) of 6.2.2;
- if  $B$  depends in a systematic way on particle concentration, extrapolate the results to infinite dilution.

## 9 Repeatability

The repeatability of the determination of average particle size, defined as  $100s_{N-1}/\langle x \rangle$ , shall be better than 5 %.

## 10 Test report

The average particle size  $x_{PCS}$  and polydispersity index PI shall be reported. These data shall be the mean and standard deviation of at least six repeated measurements on a sample and shall be calculated according to the method given in annex A. If the mean values of  $x_{PCS}$  and PI are concentration-dependent, their values extrapolated to infinite dilution, or the value obtained at the lowest concentration, shall be reported.

The following details shall also be provided:

- complete sample identification, including available information about particle shape and homogeneity;
- instrument type and number, or its detailed description if a "homemade" instrument;



NOTE 9 — The following laser source details should also be provided: type, wavelength, power and that it was vertically polarized;

c) dispersion conditions:

- dispersing liquid and its cleaning/filtering procedure,
- concentration of particulate material,
- dispersing agents and their concentration,
- dispersing procedure,
- sonication conditions: frequency and applied power (if necessary);

d) measurement conditions:

- number of concentrations investigated,
- number of particles in the measuring volume, as estimated from particle concentration and average particle size  $x_{PCS}$ ,
- maximum value of the intercept  $B_{max}$  of the autocorrelation function attainable for the given optical arrangement of the instrument.

NOTE 10 — This value can be determined with a known material (see clause 8 and annex A),

- value of the intercept  $B$  of the autocorrelation function and value of the ratio  $B/B_{max}$ ,
- count rate observed for the dispersion medium,
- viscosity and refractive index of the dispersion liquid,
- temperature of the sample,
- average count rate observed for the sample,
- number of analyses and their duration,
- the variance of the fit [see annex A, equation (A.6)];

e) if available, any other useful results or comments obtained at other scattering angles or from multi-angle results. If available, results obtained by other data analysis methods and results from other particle sizing methods;

f) analyst identification:

- name and location of laboratory,
- operator's name or initials,
- date.

A model test report form is given in annex B.

## Annex A

### (normative)

### Computation of the average particle diameter and polydispersity index

In this International Standard the two parameters describing particle size distribution, i.e. the average PCS diameter  $x_{PCS}$  and the polydispersity index PI, are determined by a variant of the so-called cumulants method<sup>[3]</sup>.

The basis for this analysis is that the experimentally recorded intensity autocorrelation function  $G_2(\tau)$  is approached by equation (C.12) of annex C. In order to obtain a linear regression, this equation is transformed as follows:

$$y(\tau) = \frac{1}{2} \ln[G_2(\tau) - A] \cong \frac{1}{2} \ln AB - \langle \Gamma \rangle \tau + \frac{\mu_2}{2} \tau^2$$

or

$$y(\tau_j) = a_0 - a_1 \tau_j + a_2 \tau_j^2 \quad (j = 1, 2, 3 \dots m) \quad \dots (A.1)$$

where  $j$  is the number of the delay channel of the correlator.

The baseline or far point  $A$  can be determined in two ways: by the total number of photon counts in a total time duration of the considered experiment, or from an estimate of  $G_2(\tau)$  for delay times  $\tau \gg 25/\langle \Gamma \rangle$ . It is recommended that both estimates of the baseline  $A$  are determined and that the largest of both is retained. However, for relative differences between the two estimates of the baseline larger than  $10^{-3}$  times the smallest value, measurement shall be discarded for further analysis.

The range of values to be retained for  $y_j = y(\tau_j)$  shall correspond to a range in  $[G_2(\tau_j) - A]$  of  $[G_2(\tau_1) - A] > [G_2(\tau_j) - A] > \{[G_2(\tau_1) - A]/100\}$  with at least one value smaller than  $\{[G_2(\tau_1) - A]/50\}$ . All values of  $[G_2(\tau_j) - A]$  in this range must be positive, otherwise the measurement shall be discarded for further analysis.

Finally, the number  $m$  of values of  $y_j$  in the acceptable range shall be at least 20.

The parameters  $a_0$ ,  $a_1$  and  $a_2$  are determined by least squares fitting of the experimental estimates of  $y(\tau_j)$  to equation (C.12) whereby the following function is minimized:

$$s(a_0, a_1, a_2) = \sum_{j=1}^m w_j (y_j - a_0 + a_1 \tau_j - a_2 \tau_j^2)^2 \quad \dots (A.2)$$

In equation (A.2) the normalized weighting factor

$$w_j = \frac{m [G_2(\tau_j) - A]^2}{\sum_{j=1}^m [G_2(\tau_j) - A]^2}$$

accounts for the nonlinear transformation of the raw data  $G_2$  into the values for  $y(\tau_j)$ .

The average PCS diameter  $x_{PCS}$  is calculated from  $a_1$  by

$$x_{PCS} = \frac{1}{a_1} \frac{kT}{3\pi\eta} \left[ \frac{4\pi n \sin(\theta/2)}{\lambda_0} \right]^2 \quad \dots (A.3)$$

where

- $k$  is the Boltzmann constant;
- $T$  is the absolute temperature;
- $\eta$  is the viscosity of the dispersion medium;
- $n$  is the refractive index of the dispersion medium;
- $\theta$  is the scattering angle;
- $\lambda_0$  is the laser wavelength *in vacuo*.

The polydispersity index PI is related to  $a_2$  and  $a_1$  by

$$PI = 2a_2/a_1^2 \quad \dots (A.4)$$

The actual value of the intercept  $B$  is computed from  $a_0$  and  $A$  by

$$B = [\exp(2a_0)]/A \quad \dots (A.5)$$

and is to be compared to the maximum value obtainable in the given experimental conditions,  $B_{\max}$ .

Measurements for which the ratio  $B/B_{\max} < 0,8$  shall be discarded.

The variance, equal to

$$s/(m-4) \quad \dots (A.6)$$

can be used as a criterion of goodness of the fit.

## Annex B (informative)

### Recommended test report form

#### B.1 Sample identification

a) Provide full sample description here: .....

.....

.....

- b) The disperse particles are:
- homogeneous spheres
  - nonhomogeneous spheres
  - unknown shape and/or homogeneity
  - nonspherical homogeneous
  - nonhomogeneous and nonspherical

#### B.2 Instrument type and number

a) Type and number  
(For "homemade" instruments, a detailed description is required.)

b) Laser source

Type: .....

Wavelength: .....nm

Power: .....mW

Polarization: vertical

Scattering angle: .....°

#### B.3 Dispersion conditions

Dispersion liquid: .....

Cleaning/filtering procedure: .....

Dispersion procedure: remarks about sample dispersion (optional), e.g.:

- sonicated or not
- appearance of precipitates or not
- .....

#### B.4 Measurement conditions

Number of concentrations: .....

Particle density (if known): .....g/cm<sup>3</sup>

Particle refractive index (if known): .....

For each concentration specify

- particle concentration No.: .....
- particle volume fraction: .....
- particle mass/unit volume: .....g/cm<sup>3</sup>

Temperature of the sample (within ± 0,3 °C): .....°C

Viscosity of the dispersion medium: .....mPa·s (or cP)

Refractive index of the dispersion medium: .....

For selected aperture or optical configuration

- measuring volume (V): .....cm<sup>3</sup>
- maximum value of the intercept  $B_{max}$ : .....

Count rate of dispersion liquid: .....kcounts/s

Sample count rate: .....kcounts/s  
(> 10 times count rate of dispersion liquid and in the range 5 kcounts/s to 1 000 kcounts/s)

Estimated number of particles in V (> 1 000): .....

Aspect of the sample (opaque samples are not allowed):

clear     blue effect     slightly turbid

Laser beam passing through the sample (no spreading halo or apparent absorbance is allowed):

sharp parallel beam

Measured value of the intercept  $B$ : .....

Ratio  $B/B_{max}$  ( $\geq 0,80$ ): .....

Optical density for 1-cm path length (if known): .....

Depolarization ratio (if known): .....

Duration of one experiment ( $\geq 60$  s): .....s

Number of experiments ( $\geq 6$ ): .....

Variance of the data-fitting: .....

Table of  $x_{PCS}$  (in nm) and PI

Run No.	$x_{PCS}$ (nm)	PI
1		
2		
3		
4		
5		
6		
.		
.		
.		

**B.5 Other results** (optional)

PCS measurements at other angles (specify): .....

Multiangle PCS results (specify the angles): .....

Results obtained by other methods (specify): .....

**B.6 Analyst identification**

Laboratory (name and place): .....

Operator (name or initials): .....

Date: .....

**B.7 Comments** (optional)

**B.8 Summary of the results**

Sample: .....

**Concentration 1**

(Mean) average PCS diameter  $x_{PCS}$ : ..... nm  
and standard deviation: ..... nm

(Mean) polydispersity index PI: .....  
and standard deviation: .....

**Concentration 2**

(Mean) average PCS diameter  $x_{PCS}$ : ..... nm  
and standard deviation: ..... nm

(Mean) polydispersity index PI: .....  
and standard deviation: .....

**Concentration ...**

If  $x_{PCS}$  and PI are concentration-dependent, give results extrapolated to infinite dilution (or result obtained at the lowest concentration)

(Mean) average PCS diameter  $x_{PCS}$  ( $\phi \rightarrow 0$ ): ..... nm  
and standard deviation: ..... nm

(Mean) polydispersity index PI ( $\phi \rightarrow 0$ ): .....  
and standard deviation: .....

## Annex C (informative)

### Theoretical background

In a typical PCS experiment, the investigated dispersion is illuminated by a narrow monochromatic and coherent source, i.e. a laser beam with one single wavelength *in vacuo*,  $\lambda_0$ . The light scattered by the dispersed particles is coherently detected at an angle  $\theta$  (typically  $90^\circ$  in this International Standard) with respect to the incident radiation. Since the dispersed particles are in continuous Brownian and/or thermal motion, the observed scattered intensity  $I(t)$  will fluctuate along the time axis. Therefore, analysis as a function of time of these intensity fluctuations provides information on the motion of the dispersed particles. In a PCS experiment, the time analysis is carried out with a correlator which constructs the time autocorrelation function  $G_2(\tau)$  of the scattered intensity:

$$G_2(\tau) = \langle I(t) \cdot I(t+\tau) \rangle \quad \dots (C.1)$$

This correlation function depends only on the time difference  $\tau$  and is independent of the arbitrary time  $t$  at which the evaluation of  $G_2(\tau)$  is started. The symbol  $\langle \dots \rangle$  refers to an average value of the product  $I(t) \cdot I(t+\tau)$  for various times  $t$ .

For a large number of monodisperse particles in Brownian motion in the measuring volume  $V$ ,  $G_2(\tau)$  is essentially an exponential decaying function of the time difference  $\tau$ :

$$G_2(\tau) = A[1 + B \exp(-2\Gamma\tau)] \quad \dots (C.2)$$

In equation (C.2),  $A$  is, in principle, a time-independent constant proportional to the square of the time-averaged scattered intensity  $\langle I \rangle$ , called baseline in this International Standard;  $B$  can be considered as an instrument factor with  $B \ll 1$ . In this International Standard it is designated as the intercept of the autocorrelation function. The decay rate  $\Gamma$  is linked to the translational diffusion coefficient  $D$  of isotropic spherical particles in Brownian motion as follows:

$$\Gamma = Dq^2 \quad \dots (C.3)$$

where  $q$  is the modulus of the scattering vector;

$$q = \frac{4\pi n}{\lambda_0} \sin(\theta/2) \quad \dots (C.4)$$

The scattering vector  $\vec{q}$  is defined as the vector difference between the incident and scattered wavevectors  $\vec{k}_i$  and  $\vec{k}_s$  respectively:  $\vec{q} = \vec{k}_s - \vec{k}_i$ . The modulus of both  $\vec{k}_i$  and  $\vec{k}_s$  are equal to  $2\pi n/\lambda_0$ , where  $n$  represents the refractive index of the suspension liquid.

Note that with PCS the diffusion coefficient  $D$  is determined and not the particle size. The latter quantity can only be determined by relating the diffusion coefficient to the particle size. For non-interacting, spherically shaped particles dispersed in a medium with viscosity  $\eta$ , the diffusion coefficient  $D$  is related to the particle diameter  $x$  by the Stokes-Einstein equation:

$$D = \frac{kT}{3\pi\eta x} \quad \dots (C.5)$$

where  $k$  is the Boltzmann constant and  $T$  the absolute temperature.

The conditions imposed by this International Standard on particle concentrations ensure that biasing effects of particle number fluctuations in the measuring volume, multiple scattering and particle interactions on particle size are avoided or at least minimized. In order to avoid bias by particle number fluctuations, this International Standard requires that at least 1 000 particles are present in the measuring volume [see 6.2.2, check 2]].

NOTE 11 — The criterion for the minimum of 1 000 particles relates to monosized materials only. For polydisperse samples a much larger number of particles smaller than  $x_{PCS}$  may be present in the measuring volume.

The biasing effects of multiple scattering (proportional to particle concentration) are minimized by setting upper limits of concentration [checks 3) to 5)]. Rather than defining a precise upper limit of concentration, these checks are designed to quantify conditions of concentration whereby multiple scattering effects are detectable. In order to avoid biasing effects of particle interaction, it is recommended to perform measurements at several concentrations and, if a systematic concentration dependence is observed, to extrapolate

the obtained results to infinite dilution (see 7.8). Although the use of previous equations is in principle limited to isotropic spherical particles, measurements on nonspherical and nonhomogeneous particles, where both translational and rotational diffusion processes are present, can be made and analyzed by these equations. In this case equations (C.3) to (C.5) and equation (C.10) define an equivalent spherical radius.

For the practical cases of polydisperse samples, equation (C.2) is written as:

$$G_2(\tau) = A[1 + Bg_1^2(\tau)] \quad \dots (C.6)$$

where the modulus of the field autocorrelation function  $g_1(\tau)$  is now related to the normalized distribution function of decay rates  $G(\Gamma)$  by:

$$g_1(\tau) = \int_0^{\infty} G(\Gamma) \exp(-\Gamma\tau) d\Gamma \quad \dots (C.7)$$

with

$$\int_0^{\infty} G(\Gamma) d\Gamma = 1$$

In equation (C.7),  $G(\Gamma)d\Gamma$  is proportional to the fraction of the intensity scattered by particles with decay rates in the range  $\Gamma$  and  $\Gamma + d\Gamma$ .

Several kinds of polydispersities may cause a distribution in decay rates:

- 1) polydispersity in size, i.e. all particles have the same shape and composition, e.g. homogeneous isotropic spheres, whereby only the linear dimensions of the individual particles differ;
- 2) polydispersity in shape, i.e. the particles differ in shape, e.g. mixtures of spheres, discs and rods, but are equal in volume and composition;
- 3) nonhomogeneous and anisotropic materials, i.e. the material is distributed differently from particle to particle, e.g. mixtures of homogeneous spheres with layered spheres;
- 4) all possible combinations of the previous kinds of polydispersity.

In the application of particle sizing by PCS, only the first kind of polydispersity is assumed.

In this International Standard, the distribution of decay rates is characterized by two parameters:

- a) the average decay rate  $\langle\Gamma\rangle$ , defined as:

$$\langle\Gamma\rangle = \int_0^{\infty} \Gamma G(\Gamma) d\Gamma \quad \dots (C.8)$$

- b) a dimensionless polydispersity index  $PI$ , i.e. a measure of the broadness of the distribution, defined as:

$$PI = \frac{\mu_2}{\langle\Gamma\rangle^2} \quad \dots (C.9)$$

where

$$\mu_2 = \int_0^{\infty} (\Gamma - \langle\Gamma\rangle)^2 G(\Gamma) d\Gamma$$

Note that for a Gaussian distribution of decay rates with mean decay rate  $\langle\Gamma\rangle$  and standard deviation  $\sigma$ , i.e.

$$G(\Gamma) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{(\Gamma - \langle\Gamma\rangle)^2}{2\sigma^2}\right]$$

the  $PI$  is related to  $\langle\Gamma\rangle$  and  $\sigma$  by:

$$PI = \frac{\mu_2}{\langle\Gamma\rangle^2} = \frac{\sigma^2}{2\langle\Gamma\rangle^2}$$

Since the Stokes-Einstein diameter is inversely proportional to the decay rate [see equations (C.3) and (C.5)], equation (C.8) is also used to define the average PCS diameter  $x_{PCS}$  in this International Standard:

$$\frac{1}{x_{PCS}} = \int_0^{\infty} \frac{1}{x} G[\Gamma(x)] d\left(\frac{1}{x}\right) \quad \dots (C.10)$$

Since in equation (C.10)  $G[\Gamma(x)]d(1/x)$  represents the fraction of the intensity scattered by particles with diameter in the range  $x$  and  $x + dx$ , the average PCS diameter  $x_{PCS}$  defined through equation (C.10) is a harmonic intensity-averaged diameter.

Note that this average diameter is in general different and larger than a weight-averaged diameter. It is also different from the average diameters determined by e.g. (low angle) laser light scattering or diffraction.

Note also that for a given size distribution,  $G(\Gamma)$  is dependent on laser wavelength and state of polarization and on the scattering angle  $\theta$  [see equations (C.3) and (C.4)]. Hence  $x_{PCS}$  and  $PI$  are for a given sample dependent on those factors. Therefore this International Standard recommends that the scattering angle be fixed at  $90^\circ$  and the use of a vertically polarized light source with fixed wavelength ( $\lambda_0 = 632,8$  nm; He-Ne laser) or other combinations of scattering angles and wavelengths keeping the ratio  $\sin(\theta/2)/\lambda_0$  constant as far as possible. For distributions with particle diameters below 30 nm there is no significant dependence of  $x_{PCS}$  and  $PI$  on laser wavelength (and on scattering angle). Therefore, for such samples requiring a more powerful laser source, this International Standard recommends the use of more powerful lasers with other wavelength (e.g. Ar-ion

lasers operating at 488 nm or 514,5 nm wavelengths) with vertically polarized radiation.

In the cumulants method<sup>[3]</sup> equation (C.7), the factor  $\exp(-\Gamma\tau)$  is expanded around  $\exp(-\langle\Gamma\rangle\tau)$ , yielding a polynomial in delay time. Truncating this polynomial at the second-order term, equation (C.6) can be approximated by:

$$G_2(\tau) = A[1 + B \exp(-2\langle\Gamma\rangle\tau) (1 + \mu_2\tau^2)] \quad \dots (C.11)$$

or by

$$G_2(\tau) = A[1 + B \exp(-2\langle\Gamma\rangle\tau + \mu_2\tau^2)] \quad \dots (C.12)$$

This equation is the basis of the determination of the average decay rate  $\langle\Gamma\rangle$ ,  $\mu_2$  and hence of the average diameter  $x_{PCS}$  and polydispersity index PI, described in annex A.

For further information see annex F.



## Annex D (informative)

### Typical PCS instruments and recommended specifications

The general set-up of PCS instruments is shown in figure D.1.

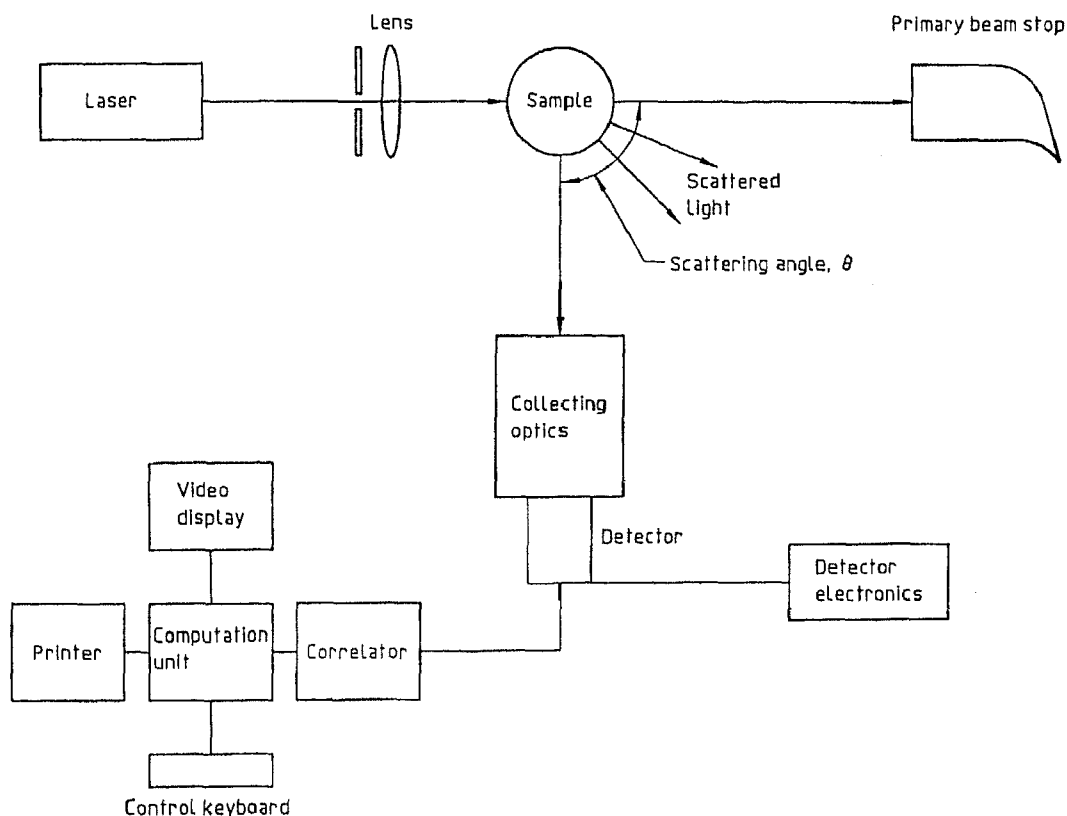


Figure D.1 — Typical set-up of a PCS instrument

In most PCS instruments a monochromatic coherent He-Ne laser with a fixed wavelength of 632,8 nm is used as the light source. Future instruments may be equipped with laser diodes operating at other wavelengths. Generally a laser power of about 2 mW to 5 mW is used. For measurements on small particles with diameters below 30 nm, the use of a laser with a higher power, such as a 15 mW to 25 mW He-Ne laser or an Ar-ion laser operating at either 488 nm or 514,5 nm wavelength, is beneficial. However, the danger of thermal convection in the sample, making data analysis uncertain, increases with laser powers above 25 mW.

A lens is often included for focusing the beam in the measuring volume. The laser radiation transmitted

through the sample is highly attenuated by the primary beam stop. Some instruments provide a measurement of the transmitted power. The radiation scattered at an angle  $\theta$  (typically  $90^\circ$ ) is collected by a detector with suitable optics. In some instruments the detector and its optics are mounted on a movable goniometer arm, allowing the scattering angle to be changed. In such instruments it is recommended that the scattering angle be set at  $90^\circ$  for a He-Ne laser source. For sources with other wavelengths, it is recommended that the angle  $\theta$  is set to fulfil the requirement that:

$$\sin[\theta/2] = \frac{\sqrt{2}}{2} \times \frac{\lambda_0}{632,8}$$

where  $\lambda_0$  is the wavelength *in vacuo* of the source, expressed in nanometres. Note that for dispersions with particles larger than roughly 300 nm and with broad size distributions, the average particle size may be dependent on the ratio  $\sin[\theta/2]/\lambda_0$ . Some instruments provide a polarization analyser before the detector.

The signal from the detector is, after amplification and discrimination, fed in digital form (as a pulse train) to a correlator unit. A computation unit usually controls the measurement and is used for storage, manipulation of the signals, and calculation of the results.

It is recommended that manufacturers provide specifications for PCS instruments on the following items. (For "homemade" instruments it is recommended that items 2) to 6) be reported.)

#### 1) General

- power requirements;
- mass;
- dimensions;
- safety and health requirements.

#### 2) Laser source

- type;
- wavelength, in nanometres;

- power, in milliwatts;
- state of polarization (only vertical polarization may be used for the purposes of this International Standard).

#### 3) Spectrometer

- scattering angle, including its range (if applicable);
- dimension of the scattering volume at 90°;
- maximum values of the intercept  $B_{\max}$  of the autocorrelation function that can be obtained.

For instruments that allow use of different detection optics conditions (e.g. the use of different apertures before the detector), it should be specified to what fixed detection condition(s) (apertures) the previous three specifications apply;

- cell type and material.

#### 4) Detector/detector electronics

- type;
- dark counts;
- minimal pulse pair resolution.

#### 5) Correlator

#### 6) Computation unit.

## Annex E (informative)

### Recommendations for sample preparation

#### E.1 General

Sample preparation consists of three parts: solvent purification, cell cleaning, and solution/suspension preparation. The first two parts are described in varying levels of detail in this section. The last part depends on the particular type of sample. Some general guidelines are given at the end of this section, and the preparation of a polystyrene latex suspension in water is described in detail.

One of the major problems in PCS measurements is dust. Dust is a general name given to any undesirable large scatterer that contributes to the signal. The presence of undesired dust may seriously bias the results for the average particle diameter.

Dust can be removed from liquids by filtration or distillation, or it can be suppressed by centrifugation. Filtration is the easiest method, thanks to the availability of inexpensive, disposable filters. Multiple filtration is the key to effectively removing dust. Multiple passes are necessary because filters do not trap all the dust on the first pass. Distillation, however, may be necessary in order to deionize the liquid and to remove trace impurities. Centrifugation requires that a suitable method for cushioning the cell is employed. This technique is used if the sample cannot be filtered; however the centrifuged dust will eventually rise up into the path of the laser beam.

The next few clauses describe several techniques for cleaning solvents. Choose the one most suitable for your particular application. Try the simplest one first. Obviously any other procedure that produces dust-free samples without altering the dispersed particles can be used.

#### E.2 Filtration

##### E.2.1 Water

To filter small quantities, use a 20 ml syringe in conjunction with a 25 mm diameter, 0,2  $\mu\text{m}$  pore size

disposable filter. Choose a syringe with a Luer-lock fitting. If a needle is required in your application, use a large diameter (18 gauge). Airborne dust is attracted to the increased surface area obtained by atomizing water through the tip of a narrow-gauge needle; therefore disperse gently, do not atomize.

Clean and flush the syringe and the needle several times to remove coarse particles which will clog a filter prematurely. If possible, use filters manufactured without adhesive residues (often polymeric coatings) which add particles to the solvent being filtered. Flush the filter several times before using to remove any such residues. Consider prefiltering if liquids are heavily laden with particles. This will extend the life of the fine-pore filters.

To filter large quantities, use a 47 mm or larger diameter filter housing with a large-area 0,2  $\mu\text{m}$  pore size filter. Attach the inlet of the filter housing to a water tap. Run at least 20 ml water through the filter in order to remove possible particles present on the filter. This flushes the residue on the surfaces of the filter, though it also reduces the filter's life. During filtration, let the water drip into the cell or dilution bottle to avoid creating a large surface area.

Figures E.1 and E.2 demonstrate these two techniques.

##### E.2.2 Organic solvents

If the solvent is compatible with the plastics filter housing and the filter material, then use the same procedures described in E.2.1 for water. If not, replace the housing with the stainless steel variety and the filter material with one suitable for use with the particular solvent. Compatibility charts are available from filter manufacturers.

To filter small quantities, use a 13 mm or 25 mm diameter stainless steel filter holder and filters in conjunction with a glass syringe. Be sure to choose O-rings that will not dissolve. Handle filters with smooth forceps to avoid puncturing them.

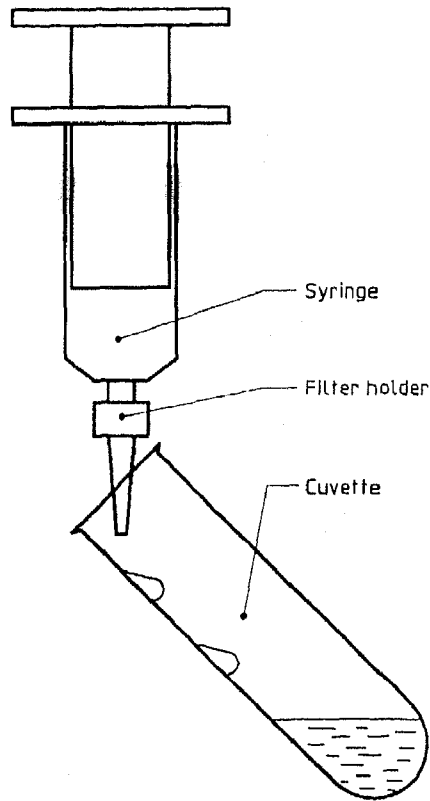


Figure E.1 — Solvent filtration, small quantities

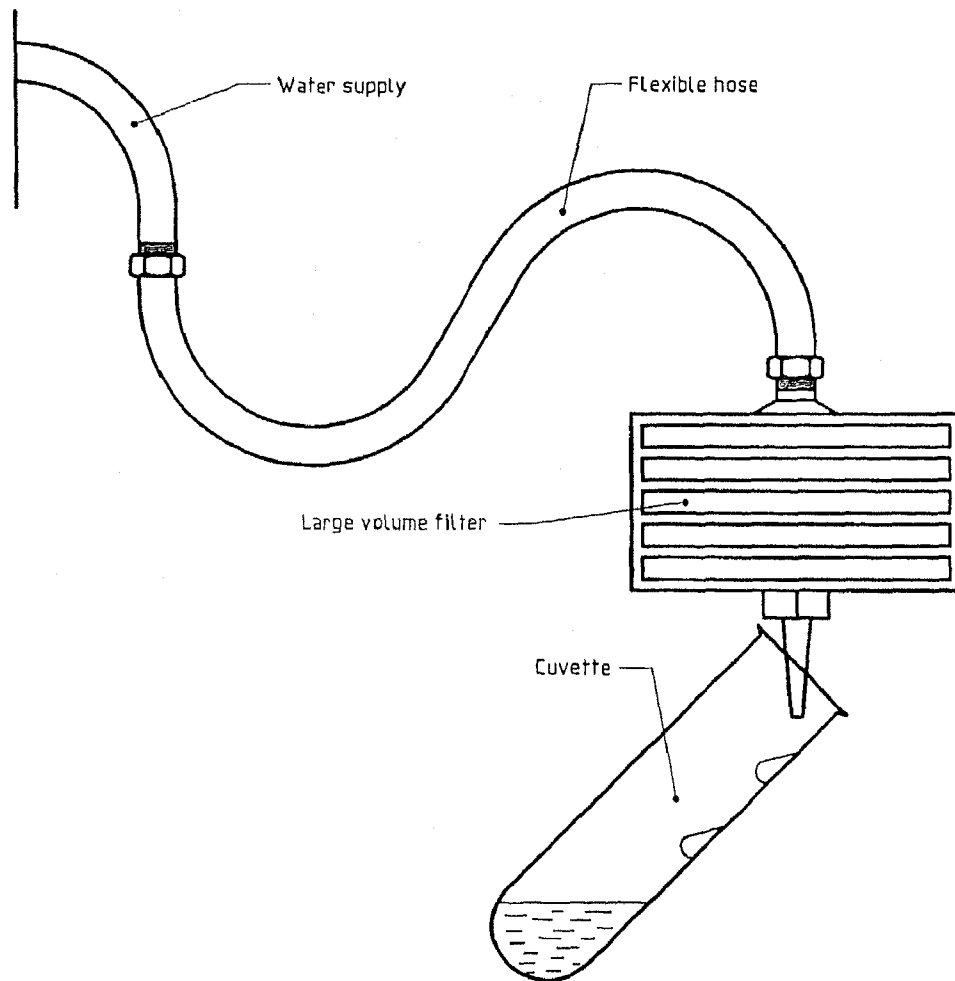


Figure E.2 — Water filtration, large quantities

## E.3 Purification

### E.3.1 Water

Dust is not the only enemy of light scattering found in liquids. Residual metal ions may affect the chemistry and shape of the scatterer. Ions may help to stabilize a sample, or they may cause flocculation. Shape may affect the diffusion coefficient and, therefore, the results of PCS measurements.

Commercial water purification systems are available from several suppliers. They consist of replaceable cartridges that remove ions, adsorb trace organic material, and filter to  $0,2 \mu\text{m}$  particle size. They are convenient, compact, safe and easy to maintain.

An alternative approach is shown in figure E.3. An ion-exchange cartridge is connected to a boiling flask/condenser followed by a collecting bottle and a  $0,2 \mu\text{m}$  pore size filter.

Use polytetrafluoroethylene (PTFE) connections throughout, except for PVC tubing on the input to the ion-exchange cartridge and on the cooling-water ports. Do not grease any of the joints.

Once assembled, run several litres of water through the system prior to use with test samples.

### E.3.2 Organic solvents

Construct a system similar to the one in figure E.3. A deionizing column is usually not required for nonpolar liquids. Replace the plastics filter housing with a 47 mm PTFE one and appropriate filters. Replace with PTFE (second choice, fluoroplastic rubber) any PVC tubing that will come into contact with the solvent. Extreme care should be taken in distilling flammable solvents.

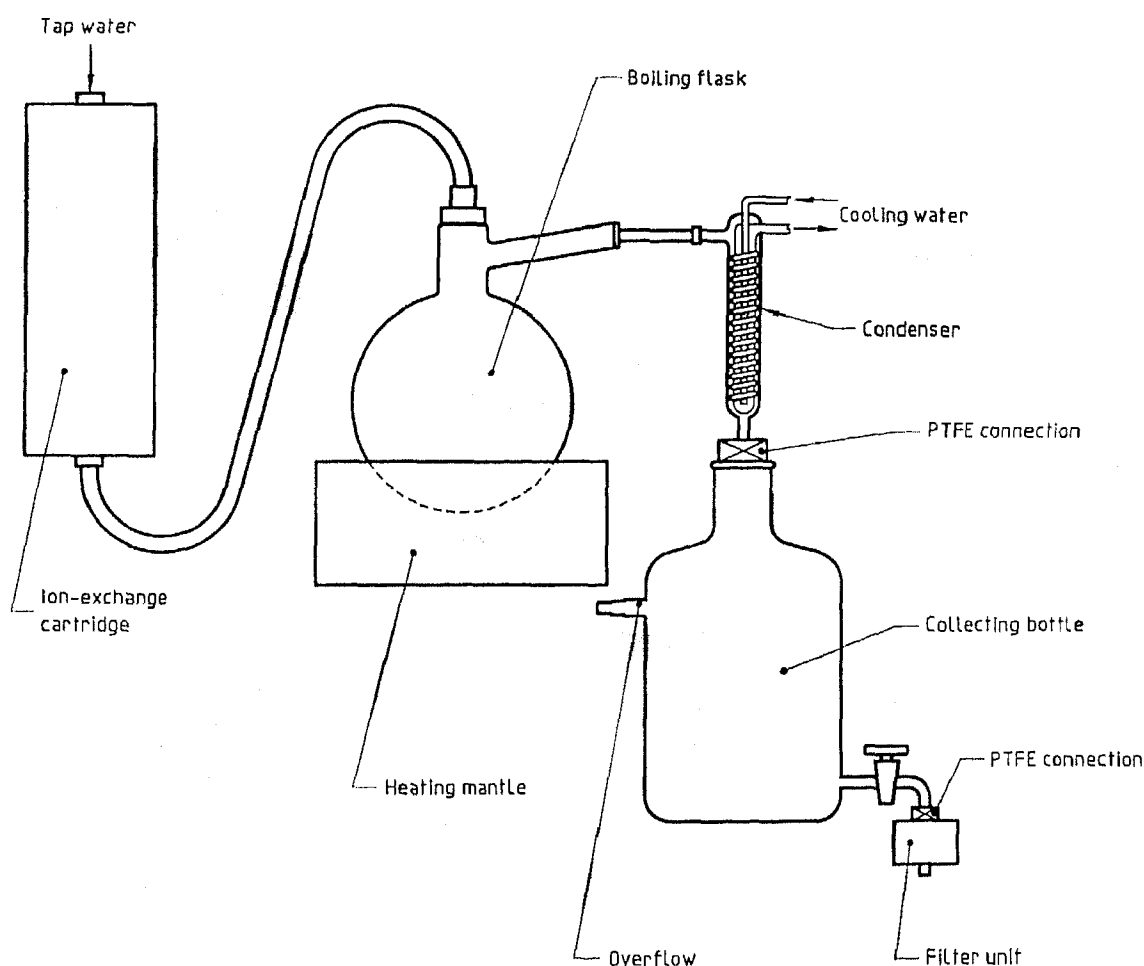


Figure E.3 — Water purification: deionization, distillation, filtration

## E.4 Cell cleaning

Like solvent purification, the degree of cell cleaning necessary depends on the application. The simplest case — for use with disposable, individually packed, clean cells — involves blowing off dust using filtered compressed air. Avoid fingerprints in the area where light enters or exits, by holding the cell near its top.

### E.4.1 Detergent and water

More rigorous cleaning procedures follow. Choose the one most suitable for your work. First, try the simplest ones. Rinse cells thoroughly in tap water. Use a nonabrasive detergent to clean the inside and the outside of the cell. Do not use a brush. Scratches of just a few micrometres can cause problems. Shake the cells containing the detergent and water vigorously instead.

To clean more thoroughly, place the cells in a small (40 W is sufficient) ultrasonic bath containing clean dilute detergent. Sonicate for several minutes. Use heat if available. To avoid scratches, prevent cells from hitting each other or the walls of the container. Change the ultrasonic cleaning liquid frequently.

Rinse thoroughly in tap water, followed by several rinses with filtered water. Let dry upside down or cover to prevent dust from collecting inside the cell.

### E.4.2 Acid

Sometimes samples adsorb onto walls of the cell. Sometimes grease spots and biological materials are not completely removed by detergent and water. If the filtered water does not dry evenly and flow in even sheets from the cell, consider the following cleaning treatment.

Soak the cell for 1 h in concentrated sulfuric acid. Use PTFE-covered tweezers to remove the cell and for further treatment. Rinse with tap water; ultrasonicate in a detergent solution; rinse several times with filtered water. Cover and dry.

If left to dry, latex samples can form a tough film on glass and quartz cells. Concentrated sulfuric acid is very effective in eliminating this film.

## E.5 Solution/suspension preparation

The most important rule is to clean everything (cells and caps, dilution bottles and caps, transfer pipettes or syringes, glassware for buffer solutions and surfactants, etc.) with purified and filtered liquid. Prepare buffer and surfactant solutions with purified and filtered liquid.

Exposure to compressed air is usually sufficient to remove dust from plastics disposable pipette tips,

but not from glass ones which, if they have been left uncovered, collect grease and dust.

To reduce the chance of reintroducing dust, minimize contact between open air and liquids used in the final phase of sample preparation. Wherever possible, use direct connections, avoiding contact with open air. Do not store filtered water for long periods. Bacteria, which scatter light nicely, grow in stored water. Prepare small amounts of fresh solutions daily if possible. If not, filter prior to use.

Where possible, avoid spraying liquids into flasks, bottles or sample cells. Allow liquid to run down the side of a clean, smooth surface; less dust is reintroduced this way.

Never shake samples violently once prepared. This may entrap dust-laden air and dissolve air in the solvent. Bubbles, too small to see, scatter more light than most particles of interest. Gentle swirling is best. Adding diluent to a concentrated drop of sample promotes uniform distribution faster than adding the drop to the diluent.

When making true molecular solutions, follow advice given in the specialized literature. Beware of gels and other supramolecular species which can dominate the light scattering and lead to false conclusions.

If particles clump together and obviously do not go into suspension, try wetting the particles with a few drops of ethanol first, followed by water.

Do not overuse surfactants. Beyond the critical micelle concentration [typically around  $10^{-4}$  % (V/V) to  $10^{-5}$  % (V/V)] a surfactant can actually promote flocculation.

## E.6 Preparing a latex suspension

For your first PCS measurement, choose a polystyrene latex of narrow particle size distribution with a diameter of about 100 nm as measured by electron microscopy or PCS. A dilute aqueous suspension of this sample will scatter very well and serve as one test of the instrument alignment. It can also be used to determine the maximum intercept  $B_{\max}$  [see clause 8 b)].

There are several sources of such polystyrene samples. Do not use samples which are more than one year old. Store these latex samples in closed vials at room temperature.

Prepare 200 ml of a solution of  $c(\text{NaCl}) = 10 \text{ mmol/l}$ . Use a disposable 0,2  $\mu\text{m}$  pore size filter to clean the liquid. Rinse thoroughly a 25 ml dilution vial and its screw-on cap with the filtered solution. Rinse a suitable sample cell and its cap. Cover both until ready for use.

The latex samples come in small, plastics squeeze-bottles. They usually contain surfactant. No more need be added. The concentration usually varies from 2 % (m/m) to 10 % (m/m) solids, in which case squeeze about four drops into the dilution vial. Add about 20 ml of the filtered 10 mmol/l NaCl solution. The result is a slightly turbid dispersion suitable for measurement with a 5 mW, He-Ne laser. If the laser is considerably more powerful, use only one drop, or dilute in two stages.

Ultrasonicate the sample in the dilution vial for a total of about 2 min using short bursts, perhaps 10 s, of sonic energy followed by a few seconds of no energy.

Clean a cell. Use the detergent-and-water technique described in E.4.1. Do not use anything abrasive. Rinse thoroughly with tap water followed by filtered

water. Rinse the cell cap. Gently pour the sample from the dilution vial into the cell. Cap it.

If wet, dry the outside of the cell prior to placing it in a sample holder. Do not wipe the cell with an abrasive towel. Pat it dry, or roll it gently on adsorbent paper, preferably one designed for use with optical-quality glass.

Place the vial in the path of the laser beam. Follow the procedures given in clause 7 of this International Standard. A distinct scattering line should be visible. If the line is thick and fuzzy, the sample is too concentrated. Dilute it. If the line is extremely weak, i.e. not much more visible than in filtered water, the sample is too dilute. Add more latex. If random bursts of light are readily apparent, prepare a new sample, cleaning the dilution vial and water more carefully.

## Annex F (informative)

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