INTERNATIONAL STANDARD

ISO 10312

Second edition
2019-10

Ambient air — Determination of asbestos fibres — Direct transfer transmission electron microscopy method

Air ambiant — Dosage des fibres d'amiante — Méthode par microscopie électronique à transmission par transfert direct

Reference number
ISO 10312:2019(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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.

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).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

This second edition cancels and replaces the first edition (ISO 10312:1995), which has been technically revised. The main changes compared to the previous edition are as follows:

— the use of electronic display systems with measurement software is permitted;

— the maximum particulate loading for TEM specimens is increased from 10 % to 25 %;

— a simplified fibre identification procedure for investigation of known sources of the regulated asbestos varieties and richterite/winchite asbestos is permitted;

— the reporting requirements have been changed to permit reporting of the concentrations of fibres and bundles longer than 5 µm and/or the concentrations of PCM equivalent fibres without the requirement to report the concentrations of structures equal to or greater than 0,5 µm;

— there is no requirement to report the 95% confidence intervals of the fibre concentrations.

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.

Introduction

This document is applicable to the determination of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation of any atmosphere. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre sizes are the relevant parameters for evaluation of the inhalation hazards, a fibre counting technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos and therefore, there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This document is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. Airborne asbestos is often found as a mixture of single fibres and more complex, aggregated structures which may or may not be also aggregated with other particles. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if a sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis would become prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of, asbestos fibres is required. The method specified in this document is designed to provide the best description possible of the nature, numerical concentration, and sizes of asbestos-containing particles found in an air sample. This document requires that a very detailed and logical procedure be used to reduce the subjective aspects of the measurement. The method of data recording specified in this document is designed to allow re-evaluation of the structure counting data as new medical evidence becomes available. All feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion onto a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. However, the procedures specified in this document are designed to minimize the disturbance of the collected particulate material, and the effect of those disturbances that do occur can be evaluated.

This document describes the method of analysis for a single air filter. However, one of the largest potential errors in characterizing asbestos in ambient atmospheres is associated with the variability between filter samples. For this reason, it is necessary to design a replicate sampling scheme in order to determine this document's accuracy and precision.

Ambient air — Determination of asbestos fibres — Direct transfer transmission electron microscopy method

1 Scope

This document specifies a reference method using transmission electron microscopy for the determination of airborne asbestos fibres and structures in in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation for asbestos structures in any atmosphere. The method allows determination of the type(s) of asbestos fibres present and also includes measurement of the lengths, widths and aspect ratios of the asbestos structures. The method cannot discriminate between individual fibres of asbestos and elongate fragments (cleavage fragments and acicular particles) from non-asbestos analogues of the same amphibole mineral[13].

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 4225, *Air quality — General aspects — Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 4225 and the following 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
acicular
shape shown by an extremely slender crystal with cross-sectional dimensions, which are small relative to its length, i.e. needle-like

3.2
amphibole
group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:

$$A_{0\text{-}1}B_2C_5T_8O_{22}(OH,F,Cl)_2$$

where

A = K, Na;

B = Fe^{2+}, Mn, Mg, Ca, Na;

C = Al, Cr, Ti, Fe^{3+}, Mg, Fe^{2+};

T = Si, Al, Cr, Fe^{3+}, Ti.

Note 1 to entry: In some varieties of amphibole, these elements can be partially substituted by Li, Pb, or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124°.

3.3
amphibole asbestos
amphibole (3.2) in an *asbestiform* (3.5) habit

3.4
analytical sensitivity
calculated airborne *asbestos structure* (3.7) concentration in structures/litre, equivalent to counting of one *asbestos* (3.6) structure in the analysis

Note 1 to entry: It is expressed in structures/litre.

Note 2 to entry: This method does not specify a unique analytical sensitivity. The analytical sensitivity is determined by the needs of the measurement and the conditions found on the prepared sample.

3.5
asbestiform
specific type of mineral fibrosity in which the *fibres* (3.22) and fibrils possess high tensile strength and flexibility

3.6
asbestos
group of silicate minerals belonging to the serpentine and *amphibole* (3.2) groups, which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong *fibres* (3.22) when crushed or processed

Note 1 to entry: The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), crocidolite (12001-28-4), grunerite asbestos (Amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4). Other varieties of asbestiform amphibole, such as richterite asbestos and winchite asbestos[19] may also be found in some products such as vermiculite and talc.

3.7
asbestos structure
individual *fibre* (3.22), or any connected or overlapping grouping of *asbestos* (3.6) fibres or bundles, with or without other particles

3.8
aspect ratio
ratio of length to width of a particle

3.9
blank
structure count made on transmission electron microscope specimens prepared from an unused filter, to determine the background measurement

3.10
camera length
equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action

3.11
chrysotile
fibrous mineral of the serpentine group, which has the nominal composition: $Mg_3Si_2O_5(OH)_4$

Note 1 to entry: Most natural chrysotile deviates little from this nominal composition. In some varieties of chrysotile, minor substitution of silicon by Al^{3+} may occur. Minor substitution of magnesium by Al^{3+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Mn^{2+} and Co^{2+} may also be present. Chrysotile is the most prevalent type of asbestos.

3.12
cleavage
breaking of a mineral along one of its crystallographic directions

3.13
cleavage fragment
fragment of a crystal that is bounded by *cleavage* (3.12) faces

Note 1 to entry: Crushing of non-asbestiform amphibole generally yields elongated fragments that conform to the definition of a fibre.

3.14
cluster
structure in which two or more *fibres* (3.22), or *fibre bundles* (3.23), are randomly oriented in a connected grouping

3.15
d-spacing
distance between identical adjacent and parallel planes of atoms in a crystal

3.16
electron diffraction
ED
technique in electron microscopy by which the crystal structure of a specimen is examined

3.17
electron scattering power
extent to which a thin layer of substance scatters electrons from their original directions

3.18
energy dispersive X-ray analysis
EDXA
measurement of the energies and intensities of X-rays by use of a solid-state detector and multi-channel analyser system

3.19
eucentric
condition when the area of interest of an object is placed on a tilting axis at the intersection of the electron beam with that axis and is in the plane of focus

3.20
field blank
filter cassette that has been taken to the sampling site, opened and then closed

Note 1 to entry: Such a filter is used to determine the background structure count for the measurement.

3.21
fibril
single *fibre* (3.22) of *asbestos* (3.6), which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances

3.22
fibre
elongated particle that has parallel or stepped sides

Note 1 to entry: For the purposes of this document, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.

3.23
fibre bundle
structure composed of parallel, smaller diameter *fibres* (3.22) attached along their lengths

Note 1 to entry: A fibre bundle may exhibit diverging fibres at one or both ends.

3.24
fibrous structure
fibre, or connected grouping of *fibres* (3.22), with or without other particles

3.25
habit
characteristic crystal growth form or combination of these forms of a mineral, including characteristic irregularities

3.26
limit of detection
calculated airborne *fibre* (3.22) concentration in structures/L, equivalent to the upper 95 % confidence limit of 2,99 structures predicted by the Poisson distribution for a count of zero structures

3.27
matrix
structure in which one or more *fibres* (3.22) or *fibre bundles* (3.23), touch, are attached to, or are partially concealed by a single particle or connected group of non-fibrous particles

3.28
Miller index
set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes

3.29
phase contrast optical microscopy equivalent fibre
PCM equivalent fibre
fibre (3.22) of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

3.30
phase contrast optical microscopy equivalent structure
PCM equivalent structure
fibrous structure (3.24) of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

3.31
pixel
smallest image-forming element to which a grey level is assigned

[SOURCE: ISO 23900-6:2015, 2.10]

3.32
primary structure
fibrous structure (3.24) that is a separate entity in the transmission electron microscope image

3.33
replication
procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made

3.34
selected area electron diffraction
SAED
technique in electron microscopy in which the crystal structure of a small area of a sample is examined

3.35
serpentine
group of common rock-forming minerals having the nominal formula:

$Mg_3Si_2O_5(OH)_4$

3.36
structure
single *fibre* (3.22), *fibre bundle* (3.23), *cluster* (3.14) or *matrix* (3.27)

3.37
twinning
occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law

3.38
unopened fibre
large diameter *asbestos* (3.6) *fibre bundle* (3.23) that has not been separated into its constituent fibrils or *fibres* (3.22)

3.39
zone-axis
line or crystallographic direction through the centre of a crystal, which is parallel to the intersection edges of the crystal faces defining the crystal zone

4 Symbols and abbreviated terms

eV	electron volt
kV	Kilovolt
l/min	litres per minute
µg	microgram (10^{-6} grams)
µm	micrometre (10^{-6} metre)
nm	nanometre (10^{-9} metre)
W	Watt
DMF	dimethylformamide
ED	electron diffraction
EDXA	energy dispersive X-ray analysis
FWHM	full width, half maximum
HEPA	high efficiency particle absolute
MEC	mixed esters of cellulose
PC	polycarbonate
PCM	phase contrast optical microscopy
SAED	selected area electron diffraction

SEM	scanning electron microscope
STEM	scanning transmission electron microscope
TEM	transmission electron microscope
UICC	Union Internationale Contre le Cancer

5 Type of sample

The method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn.

6 Range

The upper range of concentration which can be determined is 7 000 structures/mm^2 on the filter. The lower range is dependent on the area of the TEM specimens analysed, but measurement of concentrations lower than 1 structure/mm^2 can be achieved. The air concentrations represented by these values are a function of the volume of air sampled. There is no lower limit to the dimensions of asbestos fibres which can be detected. In practice, microscopists vary in their ability to detect very short asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

The method also includes provision for measurement of the concentrations of fibres with sizes thought to be of particular biological importance (fibres and bundles >5 µm), and also fibres of sizes defined in regulations (PCM equivalent fibres).

7 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of TEM specimen examined is controlled by the total suspended particulate concentration.

For total suspended particulate concentrations of approximately 10 µg/m^3, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 l of air, an analytical sensitivity of 0,5 structure/l can be obtained, equivalent to a limit of detection of 1,8 structure/l, if an area of 0,195 mm^2 of the TEM specimens is examined. For fibres longer than 5 µm, examined at lower magnifications, this limit of detection can be reduced by a further order of magnitude. If higher total suspended particulate concentrations are present, the volume of air filtered must be reduced in order to maintain an acceptable particulate loading on the filter, leading to a proportionate increase in the analytical sensitivity.

Where this is the case, lower limits of detection can be achieved by increasing the area of the TEM specimens that is examined. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre. The direct analytical method becomes increasingly difficult and imprecise as the general particulate loading of the sample collection filter increases. It is recommended that no more than approximately 25 % of the area of the grid openings be occupied by particulate that is capable of obscuring fibres of interest, which corresponds to approximately 25 µg/cm^2 of filter surface. The dimensions of the airborne particles on the filter and the dimensional range of fibres being evaluated determine the extent to which any asbestos could be overlain and obscured. If the total suspended particulate is largely organic material, the limit of detection can be lowered significantly by using an indirect preparation method.

8 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a capillary-pore polycarbonate membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,45 µm by means of a battery-powered or mains-powered pump. TEM specimens are prepared from polycarbonate filters by a carbon replication procedure[11] in which a thin film of carbon is applied to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film carbon replica of the filter surface, which bridges the openings in the TEM specimen grid, and which supports each particle from the original filter in its original position. Cellulose ester filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma[23] to ensure that all particles are exposed[12]. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure[26].

The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative structure count on randomly selected grid openings. In the TEM analysis, electron diffraction (ED)[7] is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy dispersive X-ray analysis (EDXA)[6]. For a number of reasons, it is not possible to identify each fibre unequivocally, and fibres are classified according to the techniques which have been used to identify them. A simple code is used to record, for each fibre, the manner in which it was classified. The fibre classification procedure is based on successive inspection of the morphology, the electron diffraction pattern for a selected area, and the qualitative and quantitative energy dispersive X-ray analyses. Confirmation of the identification of chrysotile is done only by quantitative ED, and confirmation of amphibole is done only by quantitative EDXA and quantitative zone axis ED.

In addition to isolated fibres, ambient air samples often contain more complex aggregates of fibres, with or without other particles. Some particles are composites of asbestos fibres with other materials. Individual fibres and structures that are more complex are referred to as “asbestos structures”. A coding system is used to record the type of fibrous structure, and to provide the optimum description of each of these complex structures. The two codes remove the requirement to interpret the structure counting data from the microscopist and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibres. The procedure permits a minimum required fibre identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fibre, and the degree of success is recorded for each fibre. The lengths and widths of all classified structures and fibres are recorded. The number of asbestos structures found on a known area of the microscope sample, together with the equivalent volume of air filtered through this area, is used to calculate the airborne concentration in asbestos structures/litre of air.

9 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water (9.1).

9.1 Water, fibre-free.

A supply of freshly distilled, fibre-free water, or another source of fibre-free, pyrogen-free water shall be used. Freshly-distilled water, filtered through a 0,1 µm pore size MEC filter, has been found satisfactory.

9.2 Chloroform.

Analytical grade, distilled in glass, preserved with a volume fraction of 1 % ethanol.

9.3 1-Methyl-2-pyrrolidone.

9.4 1,2-Diaminoethane (Ethylene diamine).

9.5 Dimethylformamide.

9.6 Glacial acetic acid.

9.7 Acetone.

10 Apparatus

10.1 Air sampling — Equipment and consumable supplies

10.1.1 Filter cassette

Field monitors, comprising 25 mm to 50 mm diameter conductive three-piece cassettes shall be used for sample collection. The cassette shall be loaded with either a capillary pore polycarbonate filter of maximum pore size 0,4 µm or an MEC or cellulose nitrate filter of maximum pore size 0,45 µm. If only fibres longer than 5 µm are to be included in the measurement, PC filters or MEC filters of maximum pore size 0,8 µm are permitted. Either type of filter shall be backed by a 5 µm pore size MEC or cellulose nitrate filter, and supported by a cellulose pad. If push-fit cassettes are used, when the filters are in position, an elastic cellulose band or adhesive tape shall be applied to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly, so that significant air leakage around the filter cannot occur.

Representative filters from the filter lot shall be analysed as specified in 12.7 for the presence of asbestos structures before any are used for air sample collection.

10.1.2 Sampling pump

The sampling pump shall be capable of a flow-rate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4,0 cm/s and 87,0 cm/s. The sampling pump used shall provide a non-fluctuating airflow through the filter, and shall maintain the initial volume flow-rate to within ±10 % throughout the sampling period. A constant flow or critical orifice-controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flow-rate of each pump is also required.

NOTE Some combinations of filter pore size and face velocity can result in distortion of the filter by the differential pressure across the filter.

10.1.3 Stand

For static sampling, a stand shall be used to hold the filter cassette at the desired height for sampling, and shall be isolated from the vibrations of the pump (10.1.2).

10.1.4 Personal sampling

For collection of air samples intended to represent the exposure of an individual, the filter cassette shall be attached within the breathing zone of the individual, i.e. within 25 cm of the worker's nose and mouth. Air sampling filter cassettes may be attached to a collar or lapel, with the open end of the cassette facing downwards.

10.1.5 Flowmeter

A calibrated flowmeter with an appropriate range and accurate to within 2,5 % of the indicated flow rate is required for calibration of the air sampling system.

Ensure that the flowmeter is clean before use in order to avoid transfer of asbestos contamination from the flowmeter to the sample being collected.

10.2 Specimen preparation laboratory

Asbestos, particularly chrysotile, may be present at trace levels in some laboratory reagents. Many building materials also contain significant amounts of asbestos or other mineral fibres which may interfere with the analysis if they are inadvertently introduced during preparation of specimens. It is most important to ensure that, during preparation, contamination of TEM specimens by any extraneous asbestos fibres is minimized. All specimen preparation steps shall therefore be performed in an environment where contamination of the sample is minimized. The primary requirement of the sample preparation laboratory is that a blank determination shall yield a result which will meet the requirements specified in 12.7. Preparation areas with filtered air are recommended. However, it has been established that work practices in specimen preparation appear to be more important than the type of clean handling facilities in use. Use of a dedicated TEM preparation area and isolation of this area from other activities such as bulk asbestos analysis is a practical way of avoiding cross contamination. Transfer of extraneous asbestos into the TEM preparation area can be avoided by the use of dedicated laboratory clothing and cleaning of the exterior surfaces of air sample cassettes prior to entry.

Preparation of samples shall be carried out only after acceptable blank values have been demonstrated.

10.3 Equipment for analysis

10.3.1 Transmission electron microscope

A TEM[3] operating at an accelerating potential of 80 kV or greater, with a resolution better than 1,0 nm, and a magnification range of approximately ×300 to ×100 000 shall be used. The ability to obtain a viewing screen magnification of about ×100 000 is necessary for inspection of fibre morphology; this magnification may be obtained by supplementary optical or electronic enlargement of the screen image. A method for making fibre length and width measurements from the screen image is required. This requirement is often fulfilled through the use of a fluorescent screen with calibrated gradations in the form of circles at 1 cm radius increments, as shown in Figure 1. This design allows lengths and widths of fibre images down to 1 mm width to be measured in increments of 1 mm, regardless of image orientation. Alternatively, electronic display systems with measurement software may be used, provided that the system is calibrated to provide fibre measurements within a variation of ±5 % at the magnification in use.

For Bragg angles less than 0,01 rad, the TEM shall be capable of performing ED from an area of 0,6 µm^2 or less, selected from an in-focus image at a screen magnification of ×20 000. This performance requirement defines the minimum separation between particles at which independent ED patterns can be obtained from each particle. If SAED is used, the performance of a particular instrument may normally be calculated using Formula (1):

$$A = 0{,}785\,4 \times \left(\frac{D}{M} + 2\,000 C_s \theta^3 \right)^2 \quad (1)$$

where

- A is the effective SAED area, in square micrometres;
- D is the diameter, in micrometres, of the SAED aperture;
- M is the magnification of the objective lens;
- C_s is the spherical aberration coefficient, in millimetres, of the objective lens;
- Θ is the maximum required Bragg angle, in radians.

